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(54) Title: METHODS AND MATERIALS FOR PREPARATION OF MODIFIED ANTIBODY VARIABLE DOMAINS AND THERAPEUTIC USES THEREOF

(57) Abstract

Methods are described for identifying the amino acid residues of an antibody variable domain which may be modified without diminishing the native affinity of the domain for antigen while reducing its immunogenicity with respect to a heterologous species and for preparing so modified antibody variable domains which are useful for administration to heterologous species. Antibody variable regions prepared by the methods of the invention are also described.

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METHODS AND MATERIALS FOR PREPARATION OF MODIFIED ANTIBODY VARIABLE DOMAINS AND THERAPEUTIC USES THEREOF

FIELD OF THE INVENTION

The present invention relates, in general, to methods for preparing a modified antibody variable domain by determining the amino acid residues of the antibody variable domain which may be modified without diminishing the native affinity of the domain for antigen while reducing its immunogenicity with respect to a heterologous species; to methods of preparation of and use of antibody variable domains having modifications at the identified residues which are useful for administration to heterologous species; and to the variable regions so modified. More particularly, the invention relates to the preparation of modified mouse antibody variable domains, which are modified for administration to humans, the resulting antibody variable domains themselves, and the use of such "humanized" antibodies in the treatment of diseases in humans.

BACKGROUND

Application of unmodified mouse monoclonal antibodies in human therapy is problematic for three reasons. First, an immune response against the mouse antibodies is mounted in the human body. Second, the antibodies have a reduced half-life in the human circulatory system. Third, the mouse antibody effector domains may not efficiently trigger the human immune system.

There are three methods which have attempted to eliminate the foregoing problems. Junghans *et al.*, *Cancer Res.*, 50, 1495-1502 (1990) and other publications

describe the utilization of genetic engineering techniques to link DNA encoding murine variable regions to DNA encoding human constant regions, creating constructs which when expressed generate a hybrid mouse/human antibody.

Also by genetic engineering techniques, the genetic information from murine hypervariable complementarity determining regions (CDRs) may be inserted in place of the DNA encoding the CDRs of a human monoclonal antibody to generate a construct encoding a human antibody with murine CDRs. This technique is known as "CDR grafting". *See, e.g.*, Jones *et al.*, *Nature*, 321, 522-525 (1986); Junghans *et al.*, *supra*.

Protein structure analysis may be used to "add back" murine residues, again by genetic engineering, to first generation variable regions generated by CDR grafting in order to restore lost antigen binding capability. Queen *et al.*, *Proc. Natl. Acad. Sci.* USA, 86, 10029-10033 (1989); Co, *et al.*, *Proc. Natl. Acad. Sci.* USA, 88, 2869-2873 (1991) describe versions of this method. The foregoing three methods are techniques to "humanize" mouse monoclonal antibodies.

As a result of the humanization of mouse monoclonal antibodies, specific binding activity of the resulting humanized antibodies may be diminished or even completely abolished. For example, the binding affinity of the modified antibody described in Queen *et al.*, *supra*, is reported to be reduced three-fold; in Co *et al.*, *supra*, is reported to be reduced two-fold; and in Jones *et al.*, *supra*, is reported to be reduced two- to three-fold. Other reports describe order-of-magnitude

reductions in binding affinity. *See, e.g.,* Tempest *et al., Bio/Technology,* 9, 266-271 (1991); Verhoeyen *et al., Science,* 239, 1534-1536 (1988).

A system for differentiating between the various subsets of T Cells, based upon cell surface antigens, is the Clusters of Differentation System (hereinafter referred to as the "CD System"). The CD System represents standard nomenclature for molecular markers of leukocyte cell differentation molecules. *See* Leukocyte Typing III White Cell Differentiation Antigens (Michael, ed. Oxford Press 1987), which is incorporated herein by reference.

So-called "pan T cell" markers (or antigens) are those markers which occur on T Cells generally and are not specific to any particular T cell subset(s). Pan T Cell markers include CD2, CD3, CD5, CD6, and CD7. The CD5 cluster antigen, for example, is one of the pan T markers present on about 85-100% of the human mature T lymphocytes and a majority of human thymocytes. CD5 is also present on a subset, about 20%, of B cells. Extensive studies using flow cytometry, immunoperoxidase staining, and red cell lysis have demonstrated that this antigen is not normally present on hematopoietic progenitor cells or on any other normal adult or fetal human tissue with the exception of the aforementioned subpopulation of B cells.

Further information regarding the CD5 marker is found in McMichael and Gotch, *in Leukocyte Typing III White Cell Differentiation Antigens (Michael, ed. Oxford Press 1987).* The CD5 molecule has also been described in the literature as reactive with

immunoglobulins. See, e.g., Kernan *et al.*, *J. Immunol.*, 33:137-146 (1984), which is incorporated herein by reference.

There are reports of attempted treatment of rheumatoid arthritis patients with monoclonal antibodies against CD4. *See* Horneff, *et al. Arthritis and Rheumatism* 34:2, 129-140 (February 1991); Goldberg, *et al.*, *Arthritis and Rheumatism*, Abstract D115, 33:S153 (September 1990); Goldberg, *Journal of Autoimmunity*, 4:617-630 (1991); Choy, *et al. Scand. J. Immunol.* 36:291-298 (1992).

There are reports of attempted treatment of autoimmune disease, particularly rheumatoid arthritis, with an anti-CD5 monoclonal antibody. *See* Kirkham, *et al.*, *British Journal of Rheumatology* 30:459-463 (1991); Kirkham, *et al.*, *British Journal of Rheumatology* 30:88 (1991); Kirkham, *et al.*, *Journal of Rheumatology* 19:1348-1352 (1992). There is also a report of an attempt to treat multiple sclerosis with an anti-T12 antibody. Hafler, *et al.* , *Neurology* 36:777-784 (1986).

As demonstrated by the foregoing, there exists a need in the art for a method of preparing antibody variable domains by identification of residues in mouse monoclonal variable region domains which may be modified without diminishing the native affinity of the domains for antigen while reducing their immunogenicity with respect to a heterologous species for use in the treatment of diseases.

Summary of the Invention

The present invention provides methods for preparing a modified antibody variable domain useful for administration to humans by determining the amino acids of a subject antibody variable domain which may be modified without diminishing the native affinity of the domain for antigen while reducing its immmunogenicity with respect to a heterologous species. As used herein, the term "subject antibody variable domain" refers to the antibody upon which determinations are made. The method includes the following steps: determining the amino acid sequence of a subject light chain and a subject heavy chain of a subject antibody variable domain to be modified; aligning by homology the subject light and heavy chains with a plurality of human light and heavy chain amino acid sequences; identifying the amino acids in the subject light and heavy chain sequences which are least likely to diminish the native affinity of the subject variable domain for antigen while, at the same time, reducing its immunogenicity by selecting each amino acid which is not in an interface region of the subject antibody variable domain and which is not in a complementarity-determining region or in an antigen-binding region of the subject antibody variable domain, but which amino acid is in a position exposed to a solvent containing the antibody; changing each residue identified above which aligns with a highly or a moderately conserved residue in the plurality of human light and heavy chain amino acid sequences if said identified amino acid is different from the amino acid in the plurality.

Another group of sequences, such as those in Figures 1A and 1B may be used to determine an alignment from which the skilled artisan may determine appropriate changes to make.

The present invention provides a further method wherein the plurality of human light and heavy chain amino acid seqeunces is selected from the human consensus sequences in Figures 5A and 5B.

In general, human engineering according to the above methods may be used to treat various diseases against which monoclonal antibodies generally may be effective. However, humanized antibodies possess the additional advantage of reducing the immunogenic response in the treated patient.

The present invention also discloses products and pharmaceutical compositions useful in the treatment of a myriad human diseases. In particular, products prepared by the foregoing methods include a modified H65 mouse monoclonal variable domain. Additionally, DNA sequences encoding the modified H65 variable domain are provided.

Modified antibody variable domains which are products of the methods of the present invention may be used, *inter alia*, as components of various immunoglobulin molecules such as Fab, Fab', and $F(ab')_2$ domains, single chain antibodies, and Fv or single variable domains.

Immunoglobulin molecules comprising modified variable domains according to the invention are particularly suited for therapeutic administration to human by themselves or, for example, as components of immunoconjugates such as those described in co-pending,

co-owned U.S. Patent Application Serial No. 07/787,567 filed on November 4, 1991.

The present invention also provides methods for treatment of autoimmune diseases, wherein animal models are predictive of the efficacy of treatment in humans. Finally, the present invention includes pharmaceutical compositions containing the humanized antibodies according to the invention are disclosed.

BRIEF DESCRIPTION OF THE DRAWING

Figures 1A and 1B are alignments of the amino acid sequences of the light and heavy chains, respectively, of four human antibody variable domains [HYH (HYHEL-10 Fab-lysosyme complex), MCPC (IgA Fab MCPC603-phosphocholine complex), NEWM (Ig Fab' NEW) and KOL (IgG1 KOL)] by criteria of sequence and structural homology;

Figure 2 is a schematic depiction of the structural relationships between the amino acid residues of the light chain of the variable domain;

Figure 3 is a schematic depiction of the structural relationships between the amino acid residues of the heavy chain of the variable domain;

Figure 4 is a schematic representation of an antibody variable domain;

Figures 5A and 5B are alignments of the consensus amino acid sequences for the subgroups of light [hK1 (human kappa light chain subgroup 1), hK3 (human kappa light chain subgroup 3), hK2 (human kappa light chain subgroup 2), hL1 (human lambda light chain subgroup 1), hL2 (human lambda light chain subgroup 2), HL3 (human

lambda light chain subgroup 3), hL6 (human lambda light chain subgroup 6), hK4 (human kappa light chain subgroup 4), hL4 (human lambda light chain subgroup 4) and hL5 (human lambda light chain subgroup 5] and heavy chains [hH3 (human heavy chain subgroup 3), hH1 (human heavy chain subgroup 1) and hH2 (human heavy chain subgroup 2)], respectively, of human antibody variable domains;

Figures 6A and 6B are alignments of human light chain consensus sequence hK1 with the actual (h65) and low-risk modified (prop) light chain sequences of the H65 mouse monoclonal antibody variable domain and of human heavy chain consensus sequence hH3 with the actual (h65) and modified (prop) heavy chain sequences of the H65 mouse monoclonal antibody variable domain, respectively;

Figures 7A and 7B are listings of the nucleotide sequences of the oligonucleotides utilized in the construction of the genes encoding modified V/J-regions of the light and heavy chains of the H65 mouse monoclonal antibody variable domain;

Figures 8A and 8B are listings of the nucleotide sequences of the genes encoding modified V/J-regions of the heavy and light chains, respectively, of the H65 mouse monoclonal antibody variable domain;

Figure 9 is a graph of the results of a competitive binding assay showing that the H65 antibody variable domain modified by a method according to the present invention retains the antigen-binding capability of the natural H65 antibody variable region;

Figures 10A and 10B are alignments of human light chain consensus hK1 and heavy chain consensus hH1 with the light and heavy chain sequences, respectively,

of the variable domain of human antibody EU, human antibody TAC, murine antibody TAC modified according to the present invention (prop) and murine antibody TAC modified according to a different method (Que);

Figure 11 is a graph of He3 IgG binding to CD5 found on Molt-4M, demonstrating that such binding is similar to that of cH65 IgG;

Figure 12 is a graph showing the effects of anti-Lyt-1 administration on the severity of collagen-induced arthritis in DBA/1J mice;

Figures 13A and 13B are depictions of human T cell recovery in spleen and blood, respectively from PBMC/SCID mice following treatment with H65 MoAb;

Figures 14A and 14B are schematic depictions of human T cell recovery in spleen and blood, respectively from PBMC/SCID mice following treatment with H65-based $F(ab')_2$ fragment;

Figure 15 is a graph of the effects of OX19 MoAb on the severity of DR BB rat collagen-induced arthritis; and

Figures 16A and 16B are alignments of human light chain consensus sequence hK1 with the actual (h65) and low and moderate risk modified (prop) light chain sequences of the H65 mouse monoclonal antibody variable domain and of human heavy chain consensus sequence hH3 with the actual (h65) and modified (prop) heavy chain sequences of the H65 mouse monoclonal antibody variable domain, respectively.

DETAILED DESCRIPTION

Methods according to the present invention include: (1) identification of the amino acid residues of

an antibody variable domain which may be modified without diminishing the native affinity of the domain for antigen while reducing its immunogenicity with respect to a heterologous species; (2) the preparation of antibody variable domains having modifications at the identified residues which are useful for administration to heterologous species; and (3) use of the humanized antibodies of the invention in the treatment of autoimmune diseases in humans. The methods of the invention are based on a model of the antibody variable domain described herein which predicts the involvement of each amino acid in the structure of the domain.

Unlike other methods for humanization of antibodies, which advocate replacement of the entire classical antibody framework regions with those from a human antibody, the methods described herein introduce human residues into the variable domain of an antibody only in positions which are not critical for antigen-binding activity and which are likely to be exposed to immunogenicity-stimulating factors. The present methods are designed to retain sufficient natural internal structure of the variable domain so that the antigen-binding capacity of the modified domain is not diminished in comparison to the natural domain.

Data obtained from the analysis of amino acid sequences of antibody variable regions using the MacImdad (Molecular Applications Group, Stanford, California) three-dimensional molecular modeling program, in conjunction with data obtained from previous theoretical studies of hypervariable region structure, and data obtained from the crystal structures of the HYH (HYHEL-10

Fab-lysosyme complex, Brookhaven structure "3HFM"), MCPC (IgA Fab MCPC603-phosphocholine complex, Brookhaven structure "2MCP"), NEWM (Ig Fab' NEW, Brookhaven structure "3FAB") and KOL (IgG1 KOL, Brookhaven structure "2IG2") antibody variable domains from the Brookhaven database (Brookhaven National Laboratory, Upton, New York), are utilized to develop the antibody variable domain model.

Figures 1A and 1B provide the sequences of the four antibody variable domains which have been crystallized. The amino acid sequences of the light and heavy chains of HYH (SEQ ID Nos. 1 and 5, respectively), MCPC (SEQ ID Nos. 2 and 6, respectively), NEWM (SEQ ID Nos. 3 and 7, respectively) and KOL (SEQ ID Nos. 4 and 8, respectively) are shown, wherein the exclamation points "!" in the MCPC light chain sequence at position 30x, the MCPC heavy chain sequence at positions 52x and 98x, the NEWM light chain at position 30x, the KOL light chain at position 93x, and the KOL heavy chain sequence at position 98x, stand for the amino acid sequences NSGNQK (SEQ ID No. 9), NKG (SEQ ID No 10), GST (SEQ ID No 11), AG, SL and HGFCSSASC (SEQ ID No 12), respectively which are variations in the length of hypervariable loop sequences among the various antibodies. Figures 2 and 3 comprise depictions of the structure of the light and heavy chains, respectively, wherein each chain is displayed "unfolded" into a flattened beta sheet structure so that interactions among the residues are easier to visualize. The strands of folded polypeptide chains are represented as thick vertical lines, connected by eight beta-turn loops. Three of the loops are

identified as antigen-binding loops or CDRs, one is accessory to the loops, and the remaining four at the "bottom" of the variable domain are not involved in antigen binding. The amino and carboxy termini of the variable domain are symbolized by small black dots at the ends of the polypeptide chains. Each amino acid position is represented as either a circle, a triangle, or a square. The covalent disulfide bond between the two cysteines at positions 23 and 88 in the light chain and the covalent disulfide bond between positions 22 and 92 in the heavy chain are each shown as a thick horizontal line. All of the residues in each chain are shown on the map, including antigen-binding residues and framework residues. The amino acid positions are numbered according to Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, Fourth Edition, U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health (1987), with the exception of those designated with a lower-case "x", which are variations in length of hypervariable loops which Kabat has numbered as "a,b,c,d....". Solid slanted lines (either single or double) connecting pairs of residues which are adjacent in three-dimensional space but not in linear sequence, represent one or two hydrogen bonds between the mutually aligned amino nitrogens and carbonyl oxygens in the backbones of the residues.

The analysis of each amino acid position to determine whether the position influences antigen binding and/or is immunogenic was based upon the information in Figures 1A, 1B, 2 and 3, as well as the additional

variable region structural information in the following paragraphs.

The basic structure of the antibody variable domain is strongly conserved. The variable domain is composed of a light chain (or subunit) and a heavy chain (or subunit), which are structurally homologous to each other and which are related by a pseudo-two-fold axis of rotational symmetry. At the "top" of the variable domain, the region farthest away from the constant domain, there are six antigen-binding loops which are built upon a larger structural framework region. The variable domain is functionally distinct from the constant domain, being connected only by two highly flexible chains and pivoting on both "ball-and-socket" joints formed by five amino acids in the heavy and light chains.

Each subunit, light or heavy, resembles a "sandwich" structure, composed of two layers of antiparallel beta pleated sheets with a propeller twist in three-dimensional space. Each amino acid chain folds back on itself repeatedly to create nine distinct strands. Three-and-one-half of these strands form the "outside" beta-sheet layer of each subunit and the other five-and-one-half form the "inside" layer. The various strands in each layer are extensively hydrogen-bonded to each other. The two beta-sheet layers within the subunit are held together by a single covalent disulfide bond and by numerous internal hydrophobic interactions. The sequences involved in bonding the strands of the subunits together are called "framework" sequences.

Certain amino acids, either in antigen-binding sequences or in framework sequences, do not actually bind antigen but are critical for determining the spatial conformation of those residues which do bind. Each antigen-binding loop requires a properly formed "platform" of buried residues, which provides a surface upon which the loop folds. One or more of the loop residues often will be buried in the platform as an "anchor" which restricts the conformational entropy of the loop and which determines the precise orientation of antigen-contacting sidechains. Thus, the shapes of the residues which make up the platform contribute to the ultimate shape of the antigen-binding loop and its affinity for specific antigens.

Amino acid sidechains exist in various different chemical environments within the subunits. Some residues are exposed to the solvent on the outer accessible surface while other residues are buried in hydrophobic interactions within a subunit. Much of the immunoglobulin variable domain is constructed from antiparallel beta pleated sheets which create amphipathic surfaces, such that the "inside" surface is hydrophobic and the "outside" surface is hydrophilic. The outside is exposed to solvent, and therefore is also exposed to the humoral environment when the domain is in the circulatory system of an animal. Amino acid sidechains which are completely exposed to the solvent and which do not physically interact with other residues in the variable domain are likely to be immunogenic and are unlikely to have any structural importance within the immunoglobulin molecule. A highly schematic representation of the

variable domain is shown in Figure 4, wherein thick lines represent peptide bonds and shaded circles denote amino acid side chains.

The two subunits of antibody variable domains adhere to each other via a hydrophobic interface region which extends along the inside beta-sheet layer from the border of the variable domain with the constant domain to the antigen-binding loops. Amino acid side chains from both subunits interact to form a three-layered "herringbone" structure. Some of these interfacial residues are components of the antigen-binding loops, and thus have a direct effect upon binding affinity. Every residue in the interface is structurally important because the conformation of the binding regions is strongly influenced by changes in the conformation of the interface.

The foregoing data and information on the structure of antibody variable domains aids in a determination of whether a particular amino acid of any variable domain is likely to influence antigen binding or immunogenicity. The determination for each amino acid position is represented by a pair of symbols (e.g., + and +, in the lines labelled "bind" and "bury", respectively) in Figures 1A, 1B, (and also in Figures 5A, 5B, 6A, 6B, 10A and 10B). In each of these pairs, the first symbol relates to antigen binding, while the second symbol relates to immunogenicity and framework structure. Tables 1, 2 and 3, below, set out the significance of the symbols and possible pairings.

Table 1

FIRST SYMBOL IN PAIR (LIGAND BINDING)

+	Little or no direct influence on antigen-binding loops, low risk if substituted
o	Indirectly involved in antigen-binding loop structure, moderate risk if changed
-	Directly involved in antigen-binding loop conformation or antigen contact, great risk if modified

Table 2

SECOND SYMBOL IN PAIR (IMMUNOGENICITY and STRUCTURE)

+	Highly accessible to the solvent, high immunogenicity, low risk if substituted
o	Partially buried, moderate immunogenicity, moderate risk if altered
-	Completely buried in subunit's hydrophobic core, low immunogenicity, high risk if changed
=	Completely buried in the interface between subunits, low immunogenicity, high risk if modified.

Table 3

SIGNIFICANCE OF PAIRS

++	Low risk	Highly accessible to the solvent and high immunogenicity, but little or no effect on specific antigen binding
o+, +o, oo	Moderate risk	Slight immunogenicity or indirect involvement with antigen binding
any - or =	High risk	Buried within the subunit core/interface or strongly involved in antigen binding, but little no immunogenic potential

The pairings set out in the Figures indicate that making mouse-to-human modifications at positions which have a pair of low risk symbols (++) (*i.e.*, a symbol in the "bind" line and a symbol in the "bury" line corresponding to one position) results in a major reduction in therapeutic immunogenicity with little chance of affecting binding affinity. At the opposite end of the spectrum, modifying positions which have a pair of high risk symbols (--) may degrade or abolish binding activity with little or no actual reduction in therapeutic immunogenicity. There are 73 low risk

positions in the variable domain (38 in the light chain and 35 in the heavy chain) which are indicated by circles in the lines labelled "risk" in Figures 1A, 1B, 5A, 5B, 6A, 6B, 10A and 10B. There are 29 moderate risk positions in the variable domain (12 in the light chain and 17 in the heavy chain) as indicated by the triangles in the lines labelled "risk" in Figs, 1A, 1B, 5A, 5B, 6A, 6B, 10A, and 10B.

The results of the above analysis may be applied to consensus sequences for the different subgroups of antibody variable domains because the structural characteristics they represent are highly conserved, even among various species. Figures 5A and 5B thus set out and align the consensus sequences (derived from Kabat *et al.*, *supra*) of the subgroups of light (hK1, SEQ ID NO: 13; hK3, SEQ ID NO: 14; hK2, SEQ ID NO: 15; hL1 SEQ ID NO: 16; hL2, SEQ ID NO: 17; hL3, SEQ ID NO: 18; hL6, SEQ ID NO: 19; hK4, SEQ ID NO: 20; hL4, SEQ ID NO: 21; and hL5, SEQ ID NO: 22) and heavy chains (hH3, SEQ ID NO: 23; hH1, SEQ ID NO: 24; and hH2, SEQ ID NO: 25) of antibody variable domains with the pairings representing the structural characteristics of each amino acid position, wherein the consensus sequences for the hL6, hK4, hL4, hL5 and hH2 subgroups were derived from less than twenty actual light or heavy chain sequences.

In the consensus sequences set out in Figures 5A and 5B, upper case amino acid designations indicate that the amino acid is present at that location in about 90% to about 100% of the known human sequences (excluding small incomplete fragments) of that subgroup (i.e., is "highly conserved"); whereas lower case amino acid

designations indicate that the amino acid is present at that location in about 50% to about 90% of the known human sequences in that subgroup (i.e., is "moderately conserved"). A lower case "x" denotes conservation in less than about 50% of the known sequences in that subgroup (i.e., a "poorly conserved" position).

The information presented in Figures 5A and 5B on the relationship of a particular amino acid in a sequence of an antibody variable domain to the structure and antigen-binding capacity of the domain is sufficient to determine whether an amino acid is modifiable. Additional structural studies, such as those on which Figures 5A and 5B are based, are not required.

Thus, according to the present invention, Figures 5A and 5B may be used to prepare, for example, a modified mouse antibody variable domain that retains the affinity of the natural domain for antigen while exhibiting reduced immunogenicity in humans by the following steps. The amino acid sequences of both the light chain and the heavy chain from the mouse variable domain are first determined by techniques known in the art (e.g., by Edman degradation or by sequencing of a cDNA encoding the variable domain). Next, the consensus sequences set out in Figures 5A and 5B for human antibody variable regions are examined to identify both a light chain consensus and a heavy chain consensus sequence that are the most homologous to the particular mouse subunit sequences that are to be modified. The mouse sequences are aligned to the consensus human sequences based on homology either by sight or by using a commercially

available computer program such as the PCGENE package (Intelligenetics, Mountain View, California).

Figures 5A and 5B are then used again to identify all of the "low risk" or "moderate risk" positions at which the mouse sequence differs significantly from the chosen human consensus. The mouse amino acid residues at these low risk and moderate risk positions are candidates for modification. If the human consensus is strongly conserved at a given low risk or moderate risk position, the human residue may be substituted for the corresponding mouse residue. If the human consensus is poorly conserved at a given low risk or moderate risk position, the mouse residue is retained at that position. If the human consensus is moderately conserved at a specific position, the mouse residue is normally replaced with a human residue, unless the mouse residue occurs at that position in at least one of the sequences (*e.g.*, in Kabat *et al.*, *supra*) on which the human consensus sequence is based. If the mouse residue does occur at that position in a human sequence then the mouse residue may be retained.

Other criteria may be important to the determination of which identified residues of a variable region are to be modified. For example, since the side chain of proline is connected to both its α-carbon and its peptide nitrogen, free rotation is restricted around the carbon-nitrogen bond (the Ramachandran ϕ angle). Therefore, wherever there is a proline in a sequence, the shape of the backbone is distorted and that distortion can influence other residues involved in antigen binding. The presence or absence of a proline residue at any point

in the amino acid sequence is a structurally important feature. If the mouse sequence contains a proline at a certain location, it is likely that its presence is necessary for a proper backbone and framework conformation and proline is perferably retained. If the mouse sequence does not contain a proline at a location where the human consensus sequence has one, it is likely that substituting a proline in the mouse sequence would affect proper conformation of the sequence, therefore the mouse residue is preferably retained. Where a proline at a particular position involving proline is changed from mouse to human, such a change is considered to be at least moderate risk even if that position would otherwise be low risk.

Similarly, insertions and deletions in a mouse sequence, relative to a human consensus framework, are normally preserved intact. If the mouse sequence has an alteration in the length and spacing of the variable region backbone, it is likely that the alteration is necessary to provide a surface for proper folding of the antigen-binding loops. The alteration is preferably retained in a modified version of the sequence.

Residues participating in the interface between the light and heavy chains of a variable domain are also preferably left intact in a modified version. They are all designated high risk, with = symbols on the "bury" lines in Figures 1, 5, 6, 10. The side chains in the interface region are buried deep within the structure, so they are unlikely to elicit a therapeutic immunogenic response in a heterologous species.

Once a modified sequence has been designed, DNAs encoding the complete variable domain are synthesized [via oligonucleotide synthesis as described, for example, in Sinha *et al.*, *Nucleic Acids Res.*, 12, 4539-4557 (1984)], assembled [via PCR as described, for example in Innis, Ed., *PCR Protocols*, Academic Press (1990) and also in Better *et al. J. Biol. Chem.* 267, 16712-16118 (1992)], cloned and expressed [via standard procedures as described, for example, in Ausubel *et al.*, Eds., *Current Protocols in Molecular Biology*, John Wiley & Sons, New York (1989) and also in Robinson *et al.*, *Hum. Antibod. Hybridomas*, 2, 84-93 (1991)], and finally tested for specific antigen binding activity [via competition assay as described, for example, in Harlow *et al.*, Eds., *Antibodies: A Laboratory Manual*, Chapter 14, Cold Spring Harbor Laboratory, Cold Spring Harbor (1988) and Munson *et al.*, *Anal. Biochem.*, 107, 220-239 (1980)].

Treatment of certain autoimmune diseases with immunotoxin conjugates is described in co-pending, commonly assigned U.S. Patent Application Serial No. 07/759,297 filed September 13, 1991, and Bernhard, *et al.*, "Materials Comprising and Methods of Preparation and Use for Ribosome-Inactivating Proteins", a United States patent application filed December 9, 1992, which are incorporated herein by reference. An immunoglobulin such as an anti-T-cell immunoglobulin may be conjugated to a cytotoxic molecule. The cytotoxic molecule to which the immunoglobulin is conjugated may be any of a number of toxins such as lectin A or a ricin A chain. The above-referenced '297 application also describes use of an

anti-CD5 antibody conjugated to a ricin A chain providing an anti-T-cell immunotoxin.

A general description of various autoimmune diseases is found in *The Autoimmune Diseases* (Rose & Mackey, eds 1985). Autoimmune diseases may be characterized, *inter alia,* by abnormal immunological regulation which results in excessive B Cell activity and diminished, enhanced, or inappropriate T Cell activity. Such altered T cell activity may result in excessive production of autoantibodies. Although the autoimmune diseases are complex and diverse in their manifestations, they possess the common feature of an impaired immune system. Therapeutic depletion of circulating T cells through the administration of an anti-pan T cell immunoglobulin improves the clinical course and prognosis of patients with autoimmune disease. For anti-CD5 antibody therapy, the additional depletion of CD5 B cells may have a further beneficial effect since CD5 B cells have been implicated in some autoimmune diseases.

Once prepared, humanized antibodies are then useful in the treatment of autoimmune disease. In this regard, an anti-CD5 monoclonal antibody is presented as an example of a preferred embodiment of the invention. An example of an anti-pan T cell immunoglobulin is an CD5 antibody which is primarily reactive with a surface antigen of mature T cells, but is also reactive with 10-20% of mature B cells. Clinical data obtained using the anti-pan T cell immunoglobulin in models of autoimmune diseases in non-human animals are predictive of the effects of using such immunoglobulins as therapy against human autoimmune diseases.

For the purpose of the present invention, an immunoglobulin, such as an antibody, is "reactive" with or "binds to" an antigen if it interacts with the antigen forms an antigen-immunoglobulin complex. The antigen is generally a unique surface protein or marker. A most preferred marker is the CD5 antigen cluster.

The anti-pan T cell immunoglobulin may be obtained from a number of sources. It is reactive with most mature T cells or with both T cells and subsets of other lymphoid cells, such as B cells or natural killer (NK) cells. The immunoglobulin may be synthetic or recombinant, including genetically-engineered immunoglobulins such as chimeric immunoglobulins, humanized antibodies, hybrid antibodies, or derivatives of any of these.

Chimeric immunoglobulins, antibodies or peptides are comprised of fused portions from different species as a product of chimeric DNA. Chimeric DNA is recombinant DNA containing genetic material from more than one mammalian species. Chimeric immunoglobulins include one portion having an amino acid sequence derived from, or homologous to, a corresponding sequence in an immunoglobulin, antibody or peptide derived from a first gene source while the remaining segment of the chain(s) is homologous to corresponding sequences of another gene source. For example, a chimeric antibody peptide may comprise an antibody heavy chain with a murine variable region and a human constant region. The two gene sources will typically involve two species, but will occasionally involve different sources from one species.

Chimeric immunoglobulins, antibodies or peptides are typically produced using recombinant molecular and/or cellular techniques. Typically, chimeric antibodies have variable regions of both light and heavy chains that mimic the variable regions of antibodies derived from one mammalian species, while the constant portions are homologous to the sequences in antibodies derived from a second, different mammalian species.

The definition of chimeric antibody, however, is not limited to this example. A chimeric antibody is any antibody in which either or both of the heavy or light chains are composed of combinations of sequences mimicking the sequences in antibodies of different sources regardless of whether these sources are differing classes, differing antigen responses, or differing species of origin, and whether or not the fusion point is at the variable/constant boundary.

The terms "humanized," "human-like" or "human-engineered" refers to an immunoglobulin wherein the constant regions have at least about 80% or greater homology to human immunoglobulin, and wherein some of the nonhuman (*i.e.* murine) variable region amino acid residues may be modified to contain amino acid residues of human origin.

Humanized antibodies may be referred to as "reshaped" antibodies. Manipulation of the complementarity-determining regions (CDRs) is one means of manufacturing humanized antibodies. *See*, e.g., Jones, *et al*. Replacing the Complementarity- Determining Regions in a Human Antibody With Those From a Mouse, *Nature*

321:522-525 (1988); Riechmann, *et al*. Reshaping Human Antibodies For Therapy, *Nature* 332, 323-327 (1988). For a review article *concerning* chimeric and humanized antibodies, see Winter and Milstein, Man-Made Antibodies, *Nature* 349, 293-299 (1991).

Preferably, immunoglobulins of the present invention are monoclonal antibodies (hereinafter referred to as "MoAbs") of the IgM or IgG isotype of murine, human or other mammalian origin. Most preferably, the MoAb is reactive with the CD5 antigen found on both T and B cells. MoAbs of other animal species may be prepared using analogous non-human mammalian markers.

A variety of methods for producing MoAbs are known in the art. *See, e.g.*, Goding, *Monoclonal Antibodies; Principles and practice* (2d ed., Academic Press 1986), which is incorporated herein by reference. Less preferred forms of immunoglobulins may be produced by methods well-known to those skilled in the art, such as by chromatographic purification of polyclonal sera to produce substantially monospecific antibody populations.

Monoclonal antibodies specifically directed against human CD5 antigen may be obtained by using combinations of immunogens and screening antigens which have only there human CD5 antigen in common or bay a screening assay designed to be specific for only anti-CD5 monoclonals. For example, production of monoclonal antibodies directed against CD5 may be accomplished by 1) immunization with human T cells expressing the CD5 antigen followed by screening of the resultant hybridomas

for reactivity against a non-human cell line transfected with human CD5 (constructed in a manner similar to that described in Nishimura, *et al., Eur. J. Immunol., 18*:747-753 (1988)); 2)immunization with a non-human cell line transfected with human CD5 followed by screening of the resultant hybridomas for reactivity against a human T cell line expressing the CD5 antigen; 3) immunization with human or non-human cell lines expressing human CD5 followed by screening of the resultant hybridomas for ability to block reactivity of existing anti-CD5 monoclonals with a human T cell line; 4) immunization with human or non-human cell lines expressing human CD5 followed by screening of the resultant hybridomas for reactivity with purified native or recombinant CD5 antigen; or 5) immunization with a recombinant derivative of the human CD5 antigen followed by screening of the resultant hybridomas for reactivity against a human T cell line expressing CD5.

A preferred monoclonal antibody for use in this invention is produced by hybridoma cell line XMMLY-H65 (H65) deposited with the American Type Culture Collection in Rockville, Maryland (A.T.C.C.) and given the Accession No. HB9286. A preferred antibody is prepared as disclosed herein using the humanized forms of the murine H65 antibody.

The generation of human MoAbs to a human antigen is also known in the art. *See, e.g.,* Koda and Glassy, *Hum. Antibod. Hybridomas,* 1(1) 15-22 (1990). Generation of such MoAbs may be difficult with conventional techniques. Thus, it may be desirable to modify the antigen binding regions of the non-human

antibodies, *e.g.*, the $F(ab')_2$ or hypervariable regions, to human constant regions (Fc) or framework regions by recombinant DNA techniques to produce substantially human molecules using general modification methods described in, for example, U.S. 4,816,397; and EP publications 173,494 and 239,400, which are incorporated herein by reference.

Alternatively, one may isolate DNA sequences which encode a human MoAb or portions thereof which specifically bind to the human T cell by screening a DNA library from human B cells according to the general protocols outlined by Huse *et al.*, *Science* 246:1275-1281 (1989), Marks, *et al.*, *J. Mol. Biol.* 222:581-597 (1991) which are incorporated herein by reference, and then cloning and amplifying the sequences which encode the antibody (or binding fragment) of the desired specificity.

In addition to the immunoglobulins specifically described herein, other "substantially homologous" modified immunoglobulins can be readily designed and manufactured utilizing various recombinant DNA techniques known to those skilled in the art. Modifications of the immunoglobulin genes may be readily accomplished by a variety of well-known techniques, such as site-directed mutagenesis. *See*, Gillman and Smith, Gene 8:81-97 (1979); Roberts, *et al.*, *Nature 328*:731-734 (1987), both of which are incorporated herein by reference. Also, modifications which affect the binding affinity of the antibody may be selected using the general protocol outlined by McCafferty, *et al.*, *Nature 348*:552-554 (1990), which is incorporated herein by reference.

In the present invention, an immunoglobulin, antibody, or peptide is specific for a T cell if it binds or is capable of binding T cells as determined by standard antibody-antigen or ligand-receptor assays. Examples of such assays include competitive assays, immunocytochemistry assays, saturation assays, or standard immunoassays such as ELISA, RIA and flow cytometric assays. This definition of specificity also applies to single heavy and/or light chains, CDRs, fusion proteins, or fragments of heavy and/or light chains, which bind T cells alone or are capable of binding T cells if properly incorporated into immunoglobulin conformation with complementary variable regions and constant regions as appropriate.

In some competition assays, the ability of an immunoglobulin, antibody, or peptide fragment to bind an antigen is determined by detecting the ability of the immunoglobulin, antibody, or peptide to compete with the binding of a compound known to bind the antigen. Numerous types of competitive assays are known and are discussed herein. Alternatively, assays which measure binding of a test compound in the absence of an inhibitor may also be used. For instance, the ability of a molecule or other compound to bind T cells can be detected by labelling the molecule of interest directly, or it may be unlabelled and detected indirectly using various sandwich assay formats. Numerous types of binding assays such as competitive binding assays are known. *See, e.g.*, U.S. Patent Nos. 3,376,110, 4,016,043; Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold

Spring Harbor Publications, N.Y. (1988), which are incorporated herein by reference.

Assays for measuring binding of a test compound to one component alone rather than using a competition assay are also available. For instance, immunoglobulins may be used to identify the presence of a T cell marker. Standard procedures for monoclonal antibody assays, such as ELISA, may be used see, Harlow and Lane, *supra*). For a review of various signal producing systems which may be used, *see* U.S. Patent No. 4,391,904, which is incorporated herein by reference.

Other assay formats may involve the detection of the presence or absence of various physiological or chemical changes that result from an antigen-antibody interaction. *See Receptor-Effector Coupling - A Practical Approach,* (Hulme, ed., IRL Press, Oxford 1990), which is incorporated herein by reference.

Humanized antibodies of the present invention may be administered to patients having a disease having targetable cellular markers. Such disease include, but are not limited to , autoimmune diseases such as lupus (including systemic lupus erythematosus and lupus nephritis), scleroderma diseases (including lichen sclerosis, morphea and lichen planus), rheumatoid arthritis and the spondylarthropathies, thyroiditis, pemphigus vulgaris, diabetes mellitus type 1, progressive systemic sclerosis, aplastic anemia, myasthenia gravis, myositis including polymyositis and dermatomyositis, Sjogren's disease, collagen vascular disease, polyarteritis, inflammatory bowel disease (including Crohn's disease and ulcerative colitis), multiple

sclerosis, psoriasis and primary biliary cirrhosis; diseases caused by viral infections; diseases caused by fungal infections; diseases caused by parasites; and the like.

Immunoglobulins, antibodies or peptides according to the invention may be administered to a patient either singly or in a cocktail containing two or more antibodies, other therapeutic agents, compositions, or the like, including, but not limited to, immunosuppressive agents, potentiators and side-effect relieving agents. Of particular interest are immunosuppressive agents useful in suppressing allergic or other undesired reactions of a host. Immunosuppressive agents include prednisone, prednisolone, dexamethasone, cyclophosphamide, cyclosporine, 6-mercaptopurine, methotrexate, azathioprine, and gamma globulin. All of these agents are administered in generally accepted efficacious dose ranges such as those disclosed in the *Physician's Desk Reference,* 41st Ed. (1987). In addition to immunosuppressive agents, other compounds such as an angiogenesis inhibitor may be administered with the anti-pan T immunoglobin. *See* Peacock, *et al., Arthritis and Rheum.* 35 (Suppl.), Abstract, for ACR meeting No. B141 (Sept. 1992).

In a preferred embodiment of the present invention, anti-pan T cell immunoglobulins may be formulated into various preparations such as injectable and topical forms. Parenteral formulations are preferred for use in the invention, most preferred is intramuscular (i.m.) or intravenous (i.v.) administration. The formulations containing therapeutically effective amounts

of anti-pan T cell antibodies are either sterile liquid solutions, liquid suspensions or lyophilized versions and optionally contain stabilizers or excipients. Lyophilized compositions are reconstituted with suitable diluents, e.g., water for injection, saline, 0.3% glycine and the like, at a level of from about .01 mg/kg of host body weight to about 10 mg/kg or more of host body weight.

Typically, the pharmaceutical compositions containing anti-pan T cell immunoglobulins are administered in a therapeutically effective dose in a range of from about 0.01 mg/kg to about 5 mg/kg body weight of the treated animal. A preferred dose range of the anti-pan T cell antibody is from about 0.05 mg/kg to about 2 mg/kg body weight of the treated animal. The immunoglobulin dose is administered over either a single day or several days by daily intravenous infusion. For example, for a patient weighing 70 kg, about 0.7 mg to about 700 mg per day is a preferred dose. A more preferred dose is from about 3.5 mg to about 140 mg per day.

Anti-pan T cell immunoglobulin may be administered systemically by injection intramuscularly, subcutaneously, intrathecally, intraperitoneally, into vascular spaces, or into joints (e.g., intraarticular injection at a dosage of greater than about 1 μ g/cc joint fluid/day). The dose will be dependent upon the properties of the anti-pan T cell immunoglobulin employed, e.g., its activity and biological half-life, the concentration of anti-pan T cell antibody in the formulation, the site and rate of dosage, the clinical tolerance of the patient involved, the autoimmune disease

afflicting the patient and the like as is well within the knowledge of the skilled artisan.

The anti-pan T cell immunoglobulin of the present invention may be administered in solution. The pH of the solution should be in the range of about pH 5.0 to about 9.5, preferably pH 6.5 to 7.5. The anti-pan T cell immunoglobulin or derivatives thereof should be in a solution having a pharmaceutically acceptable buffer, such as phosphate, tris (hydroxymethyl) aminomethane-HCl, or citrate and the like. Buffer concentrations should be in the range from about 1 to about 100 mM. A solution containing anti-pan T cell immunoglobulin may also contain a salt, such as sodium chloride or potassium chloride in a concentration from about 50 to about 150 mM. An effective amount of a stabilizing agent such as albumin, a globulin, a detergent, a gelatin, a protamine, or a salt of protamine may also be included and may be added to a solution containing anti-pan T cell immunoglobulin or to the composition from which the solution is prepared. Systemic administration of anti-pan T cell immunoglobulin is typically made every two to three days or once a week if a chimeric or humanized form is used. Alternatively, daily administration is useful. Usually administration is by either intramuscular injection or intravascular infusion.

Alternatively, anti-pan T cell immunoglobulin is formulated into topical preparations for local therapy by including a therapeutically effective concentration of anti-pan T cell immunoglobulin in a dermatological vehicle. Topical preparations may be useful to treat skin lesions such as psoriasis and dermatitis associated

with lupus. The amount of anti-pan T cell immunoglobulin to be administered, and the anti-pan T cell immunoglobulin concentration in the topical formulations, will depend upon the vehicle selected, the clinical condition of the patient, the systemic toxicity and the stability of the anti-pan T cell immunoglobulin in the formulation. Thus, the physician will necessarily employ the appropriate preparation containing the appropriate concentration of anti-pan T cell immunoglobulin in the formulation, as well as the amount of formulation administered depending upon clinical experience with the patient in question or with similar patients.

The concentration of anti-pan T cell immunoglobulin for topical formulations is in the range from about 0.1 mg/ml to about 25 mg/ml. Typically, the concentration of anti-pan T cell immunoglobulin for topical formulations is in the range from about 1 mg/ml to about 20 mg/ml. Solid dispersions of anti-pan T cell immunoglobulin as well as solubilized preparations may be used. Thus, the precise concentration to be used in the vehicle may be subject to modest experimental manipulation in order to optimize the therapeutic response. Greater than about 10 mg of anti-pan T cell immunoglobulin/100 grams of vehicle may be useful with 1% w/w hydrogel vehicles in the treatment of skin inflammation. Suitable vehicles, in addition to gels, are oil-in-water or water-in-oil emulsions using mineral oils, petrolatum, and the like.

Anti-pan T cell immunoglobulin may be optionally administered topically by the use of a transdermal therapeutic system (Barry, *Dermatological*

Formulations, p. 181 (1983)). While such topical delivery systems have been designed largely for transdermal administration of low molecular weight drugs, by definition they are capable of percutaneous delivery. They may be readily adapted to administration of anti-pan T cell immunoglobulin or derivatives thereof and associated therapeutic proteins by appropriate selection of the rate-controlling microporous membrane.

Preparations of anti-pan T cell immunoglobulin either for systemic or local delivery may be employed and may contain excipients as described above for parenteral administration and other excipients used in a topical preparation such as cosolvents, surfactants, oils, humectants, emollients, preservatives, stabilizers and antioxidants. Any pharmacologically acceptable buffer may be used, e.g., tris or phosphate buffers.

Administration may also be intranasal or by other nonparenteral routes. Anti-pan T cell immunoglobulin may also be administered via microspheres, liposomes or other microparticulate delivery systems placed in certain tissues including blood.

Anti-pan T cell immunoglobulin may also be administered by aerosol to achieve localized delivery to the lungs. This is accomplished by preparing an aqueous aerosol or liposomal preparation. A nonaqueous (e.g., fluorocarbon propellent) suspension may be used. Sonic nebulizers preferably are used in preparing aerosols. Sonic nebulizers minimize exposing the anti-pan T cell antibody or derivatives thereof to shear, which can result in degradation of anti-pan T cell immunoglobulin.

Ordinarily, an aqueous aerosol is made by formulating an aqueous solution or suspension of anti-pan T cell immunoglobulin together with conventional pharmaceutically acceptable carriers and stabilizers. The carriers and stabilizers will vary depending upon the requirements for the particular anti-pan T cell immunoglobulin, but typically include nonionic surfactants (Tweens, Pluronics, or polyethylene glycol), innocuous proteins such as serum albumin, sorbitan esters, oleic acid, lecithin, amino acids such as glycine, buffers, salts, sugars, or sugar alcohols. The formulations are sterile. Aerosols generally may be prepared from isotonic solutions.

Each of the foregoing methods are illustrated by way of the following examples, which are not to be construed as limiting the invention. All references cited herein are incorporated by reference.

EXAMPLES

EXAMPLE 1

A. Identification of Low Risk Residues in A Mouse Variable Domain

A method of the present invention was utilized to prepare modified antibody variable domains by identifying low risk residues in a mouse monoclonal antibody variable domain, designated H65, which may be modified without diminishing the native affinity of the domain for antigen while still reducing its immunogenicity with respect to humans.

The light and heavy chains of the variable domain of H65 were determined to most closely resemble the consensus sequences of subgroup 1 ("hK1") of the human kappa chains and subgroup 3 ("hH3") of the human heavy chains, respectively. The H65 V/J-segments of the light and heavy chain sequences are aligned with the two human subgroup consensus sequences in Figures 6A and 6B. The H65 sequences are also contained in SEQ ID Nos. 26 and 28.

In Figures 6A and 6B, upper and lower case letters denote the degree of conservation at any given position. For example, an "A" indicates that alanine is present at that position in about 90% to about 100% of the known human sequences of that subgroup (excluding small, incomplete fragments); whereas an "a" indicates that alanine is present only about 50% to about 90% of the time at that position in known human sequences of that subgroup. A lower case "x" indicates conservation of the amino acid at that position less than about 50% of the time.

The line labelled "bind" shows which residues directly affect (-) or do not directly affect (+) antigen binding of CDR loops. The "bury" line indicates exposed (+), buried (-), or interfacial (=) residues. On either the "bind" or "bury" line, a "0" indicates a residue of intermediate significance in terms of antigen binding or placement of the residue, respectively.

Figures 6A and 6B reveal that the mouse H65 sequences differ from the human consensus sequences with which they are aligned at a total of 94 positions. Sixty-nine of these differences occur at moderate-risk

(15 positions) or high risk (54 positions) positions suggesting that the mouse residue at that position may be important for the function of the antibody. The "M/H" line of Figure 6 specifically indicates which positions differ between the two pairs of aligned sequences. Based on the considerations of the level of risk and the degree of conservation of the human residue at each position presented in the foregoing paragraphs, those residues in the H65 sequences designated M or m in the M/H line are identified as residues to be kept "mouse" in a humanized sequence, while those designated H or h are identified as residues to be changed to "human."

Twenty-five differences occur at low risk positions at which the mouse and human sequences differ. At thirteen of those positions (designated "H" on the M/H lines of Figure 6) the mouse residue aligns with a human consensus amino acid which is highly conserved. Therefore, the mouse residue at that position is identified as one to be changed to the conserved human residue.

At four low risk positions (designated "m") in which the mouse and the human sequences differ, the mouse residue aligns with a human consensus amino acid which is moderately conserved. However, since the mouse residue is found at that position in other actual sequences of human antibodies (in Kabat's sequences of Proteins of Immunoglobulin Interest), the positions are identified as ones to be kept "mouse." At seven low risk positions (designated "h"), the mouse residue aligns with a human consensus amino acid which is moderately conserved but the mouse residue is not found at that position in an

actual human antibody sequence in the Kabat book. Therefore, those positions are identified as ones to be changed to "human."

At one low risk position (designated "m") in which the mouse and human sequences differ, the mouse residue aligns with a human consensus amino acid which is poorly conserved. Therefore, that position is identified as one to be kept "mouse."

The "prop" lines of Figure 6 set out the sequences of the light and heavy chains of the H65 antibody variable domain in which the residues identified by the methods of the present invention as those which may be modified without diminishing the native affinity of the H65 variable domain for CD5 are changed to human residues. Thus, the "prop" lines of Figures 6A and 6B set out the amino acid sequences of humanized light (SEQ ID NO: 27) and heavy chains (SEQ ID NO: 29) of the H65 antibody variable domain.

EXAMPLE 2

A. Synthesis of H65 V/J Segments of light and heavy chain

Based on the low risk humanized amino acid sequences of the V/J-segments of the light and heavy chains of the H65 antibody variable domain described in Example 1, synthetic genes for heavy and light chain V/J-segments of H65 were synthesized. The humanized amino acid sequences were reverse-translated with the PCGENE package (Intelligenetics, Mountain View, California). Amino acid codons for each position were chosen which were identical to the mouse codon at

positions where the mouse amino acid residue was maintained, or which matched as closely as possible a codon in a native antibody gene based on those gene sequences published in Kabat et al, *supra*. For expression of humanized whole antibody in mammalian cells, polynucleotides encoding the native mouse leader sequences were included as part of the humanized genes. Each gene, heavy or light, was assembled from six overlapping oligonucleotides and amplified by PCR. Each oligonucleotide was synthesized with a Cyclone Model 8400 DNA Synthesizer (Milligen/Biosearch, Burlington, Massachusetts). Restriction sites were introduced into the amplified DNA segments for cloning into the final expression vectors for antibody genes (heavy or light). A SalI restriction site was introduced into each V-region upstream of the initiation codon, ATG. A BstEII restriction site was introduced into the 3'-end of the heavy chain J-region, while a HindIII site was introduced into the 3'-end of the light chain J-region.

B. Construction of the Gene Encoding the Humanized H65 Heavy Chain Variable Region

The humanized V- and J-segments of the heavy chain were assembled from six oligonucleotides, HUH-G1, HUH-G2, HUH-G3, HUH-G4, HUH-G5, and HUH-G6, the sequences of which are contained in Figure 7B and in SEQ ID Nos. 36 to 41, respectively. The oligonucleotides were amplified with PCR primers H65G-2S and H65-G2 (SEQ ID Nos. 42 and 43, respectively). Oligonucleotides greater than 50 bp in length were purified on a 15% polyacrylamide gel in the presence of 25% urea. DNA strand extension and DNA

amplification was accomplished with a Taq polymerase and the GeneAmp Kit used according to the manufacturer's instructions (Perkin-Elmer Cetus, Germany). Oligonucleotides containing the synthetic humanized antibody gene were mixed in pairs (HUH-G1 + HUH-G2, HUH-G3 + HUH-G4, and HUH-G5 + HUH-G6) in 100 μl reactions with 1 μg of each DNA, 2.5 U Taq polymerase, 50 mM KCl, 10 mM TRIS-Cl pH 8.3, 1.5 mM $MgCl_2$, and 200 uM each dNTP. The tube was incubated in a Coy TempCycler for 1 minute at 94°C, 2 minutes at 55°C and 20 minutes at 72°C. A portion of each reaction product (40 μl) was mixed in pairs (HUH-G1,2 + HUH-G3,4; HUH-G3,4 + HUH-G5,6), 2.5 U Taq was added and the tubes were re-incubated at 94°C for 1 minute, 55°C for 2 minutes and 72°C for 20 minutes. The heavy chain gene was then assembled by mixing an equal amount of the HUH-G1,2,3,4 reaction product with the HUH-G3,4,5,6 reaction product and bringing the volume to 100 μl of 2.5 U Taq, 50 mM KCl, 10 mM TRIS-Cl pH 8.3, 1.5 mM $MgCl_2$, 200 uM each dNTP, and 0.5 μg of each amplification primer H65G-2S and H65-G2. The reaction was overlaid with mineral oil, and the cycle profile used for amplification was: denaturation 94°C for 1 minute, annealing 55°C for 2 minutes, and primer extension at 72°C for 3 minutes. Primer extension was carried out for 30 cycles. The DNA sequence of the assembled V/J-region is contained in Figure 8A and in SEQ ID NO: 46. The assembled V/J-region was cut with SalI and BstEII, purified by electrophoresis on an agarose gel, and assembled into a heavy chain expression vector, pING4612, which is similar to that described for heavy chain expression in Robinson *et al., Hum. Antib. Hybridomas, 2*

, 84 (1991) and described in detail in co-pending, co-owned U.S. Patent Application Serial No. 07/659,409 filed on September 6, 1989, which is incorporated herein by reference.

C. Construction of the Gene Encoding the Humanized H65 Light Chain Variable Region

The humanized V- and J-segments of the light chain were also assembled from six oligonucleotides, $H65K-1, HUH-K1, HUH-K2, HUH-K3, HUH-K4 and HUH-K5, the sequences of which are contained in Figure 7 and in SEQ ID NOs. 30 to 35, respectively. The oligonucleotides were amplified with PCR primers H65K-2S and JK1-HindIII (SEQ ID NOs. 44 and 45, respectively). Oligonucleotides containing the synthetic humanized antibody gene were mixed in pairs ($H65K-K1 + HUH-K1, HUH-K2 + HUH-K3, and HUH-K4 + HUH-K5) and incubated as described above for the heavy chain. A portion of each reaction product (40 μl) was mixed in pairs ($H65K-1/HUH-K1 + HUH-K2,3; HUH-K2,3 + HUH-K4,5) and treated as above. The light chain gene was then assembled by amplifying the full length gene with PCR primers H65K-2S and JK1-HindIII as outlined above for the heavy chain. The DNA sequence of the assembled V/J-region is contained in Figure 8B and in SEQ ID NO. 47. The assembled V/J-region was cut with SalI and HindIII,

purified by electrophoresis on an agarose gel, and assembled into a light chain antibody expression vector, pING4614 similar to those described for light chain expression in Robinson *et al.*, *supra*. and in U.S. Patent Application No. 07/659,409, *supra*.

D. Transient Expression of Humanized H65 IgG

Expression vectors containing the humanized H65 light chain and heavy chain sequences under the control of the Abelson Leukemia virus LTR promoter (described in Robinson *et al.*, *supra*, and in U.S. Patent Application No. 07/659,409, *supra*) and 3' untranslated regions from human gamma-1 (for heavy chain) and mouse kappa (for light chain) were transfected by lipofection into a CHO-K1 strain which expresses the SV40 T antigen. Following treatment with lipofection reagent (Bethesda Research Labs, Gaithersburg, Maryland) plus DNA for 5 hours at 37°C, Ham's F12 media containing fetal bovine serum (FBS, final FBS conc. = 10%) was added and the cells were incubated for an additional 48 hours. Following this incubation period, the FBS-supplemented media was removed and replaced with serum-free media (HB-CHO) (Irvine Scientific, Irvine, California) and the

cells were incubated for an additional 7 days. As a control, the CHO-K1 cells were also transfected with chimeric H65 light chain and heavy chain (each consisting of unmodified mouse V/J-segments fused to a human C-segment) in expression vectors similar to those described above. Following incubation, the supernatants were collected and tested by ELISA for the presence of secreted IgG. All of the supernatants contained about 0.03-0.06 μg/ml IgG.

EXAMPLE 3

The H65 antibody modified according to the methods of the present invention was tested to determine whether it retained native affinity for antigen. Its binding capability was compared to that of a chimeric H65 IgG antibody (consisting of the chimeric H65 light chain and heavy chain described in Example 2) which has the same affinity for CD5 as unmodified H65 mouse antibody.

A. Preparation of Humanized and Chimeric H65 IgG for Competition Binding

The humanized H65 (hH65) and chimeric H65 IgG (cH65) from transient transfections described above were concentrated from 4 ml to a final volume of 100 μl by centrifugation using a Centricon 30 (Amicon, Amicon

Division of W.R. Grace and Co., Beverley, Massachusetts) at 4°C. Both hH65 and cH65 concentrates were then washed once with 1.0 ml of phosphate buffered saline (PBS), pH 7.2 and reconcentrated to approximately 100 µl. As a control, HB-CHO culture media alone (CM) or media supplemented with purified cH65 (CM+cH65) was concentrated in a similar manner. The final concentrations of hH65 and cH65 were determined by ELISA (anti-human Kappa pre-coat, peroxidase-labelled anti-human gamma for detection) using chimeric IgG as a standard.

B. Radiolabelling of cH65 IgG

20 µg of purified cH65 IgG was iodinated (1 mCi of $Na^{125}I$, Amersham, Arlington Heights, Illinois) using lactoperoxidase beads (Enzymobeads, BioRad Laboratories, Richmond, California) in PBS. Iodination was allowed to proceed for 45 minutes at 23°C. ^{125}I-cH65 IgG was purified from unbound ^{125}I by gel filtration using a Sephadex G-25-80 column. Concentration and specific activity was determined by measuring the TCA-precipitated counts before and after purification.

C. Competitive Binding of hH65 for cH65 IgG

Molt4-M cells, which express CD5 on their surface, were plated into 96 well V-bottom plates at a density of 3 X 10^5 cells per well and pelleted by centrifugation. The medium was decanted, and 100 µl of purified cH65 IgG at final concentrations from 200 nM to 0.0017 nM (diluted in 3-fold steps) in "BHD" [DMEM (Dulbecco's Modified Eagle's Medium) + 1% BSA + 10 mM

Hepes, pH 7.2] (BHD) was added to each well, followed by 100 μl of ^{125}I-cH65 IgG (final concentration = 0.1 nM) in BHD. For single point determinations, 50-100 μl of the Centricon® concentrates were added to the wells as follows: hH65 (final concentration = 0.54 nM), cH65 (final concentration = 0.22 nM), CM + purified cH65 IgG (final concentration = 30 nM) and CM alone. These were followed by addition of ^{125}I-cH65 IgG (final concentration = 0.1 nM). Binding was allowed to proceed for 5 hours at 4°C. At the end of 5 hours, binding was terminated by three washes with ice cold BHD using centrifugation to pellet cells. Radioactivity was determined by solubilizing bound ^{125}I-cH65 IgG with 1N NaOH and counting in a Beckman Gamma 8000 (Beckman Instruments, Fullerton, California).

Purified cH65 IgG effectively displaced ^{125}I-cH65 IgG binding with an ED_{50} of approximately 1.0 nM as shown in Figure 9, wherein open circles indicate cH65, shaded squares indicate hH65 and shaded triangles indicate CM + purified cH65. The hH65 was as effective in displacing ^{125}I-cH65 IgG as were purified cH65 and CM + purified cH65 IgG, at their respective concentrations. No competition was observed with CM as expected. These results demonstrate that the low-risk changes made in the course of modification of hH65 did not diminish the binding affinity of this antibody for the CD5 antigen.

EXAMPLE 4

The method of the present invention for preparing modified antibody variable domains by identifying modifiable amino acids was applied to the

anti-TAC antibody variable domain sequence [SEQ ID Nos. 49 (light chain) and 53 (heavy chain)] and the resulting modified sequence is compared to the humanized anti-TAC antibody sequence [SEQ ID Nos. 51 (light chain) and 55 (heavy chain)] described in Queen *et al., supra*.

The results are shown in Figures 10A and 10B. The sequence modified according to the present invention [SEQ ID Nos. 50 (light chain) and 54 (heavy chain)] is shown on the lines labelled "prop," and the Queen humanized sequence is shown on lines labelled "Que." Modifications to the Queen humanized sequence were based on the human EU antibody sequence [SEQ ID Nos. 48 (light chain) and 52 (heavy chain)]. The comparison reveals many differences between the proposed sequence generated by the methods of the present invention and the Queen humanized sequence. The differences which are the most likely to affect binding activity of their humanized antibody are positions 4 (L vs. M), 15 (P vs. V), 36 (F vs. Y), 47 (W vs. L), 71 (Y vs. F), and 80 (A vs. P) in the light chain, as well as position 69 (L vs. I) in the heavy chain.

EXAMPLE 5

Active Modified Antibodies May Be Evolved Toward Human

If it is desirable to humanize an antibody variable domain beyond the changes identified above, further, higher-risk changes may be made to evolve the domain.

Higher-risk residues may be changed in a round of mutagenesis subsequent to the low risk changes, in smaller groups, so that deleterious mutations may be

identified quickly and corrected before binding activity is abolished. (Low risk changes can be made all at once, with little fear of abolishing activity.)

For example, because in the three-dimensional model of each subunit, framework 1 and framework 3 (F1 and F3 in Figures 2 and 3) form semi-independent loops on the surface of the subunit, the moderate or high risk mutations may therefore be divided into four groups (consisting of F1 and F3 in the light subunit and F1 and F3 in the heavy subunit). Four different constructs may be made, each containing higher-risk "human" mutations in only one framework region with the other three frameworks left completely "mouse," and assayed for activity. This technique avoids the dilemma raised by other humanization methods in which all higher-risk changes are made at once, making it difficult to determine which of the many amino acid changes is responsible for affecting antigen-binding activity. The creation of antibodies according to the invention which possess moderate risk changes are described below.

EXAMPLE 6

Identification of Moderate Risk Residues in Mouse Variable Domain

The human consensus sequences in which moderate risk residues are converted from mouse residues to human residues are represented in Figures 16A and 16B as lines labelled hK1 (*i.e.*, subgroup 1 of the human kappa chain) and hH3 (*i.e.*, subgroup 3 of the human heavy chain). Symbols in this Figure, for conservation and for risk are used in accordance with Figures 6A and 6B.

In the line labelled "mod", a dot (.) represents a residue which may be mutated from "mouse" to "human" at moderate risk. There are 29 such moderate risk positions.

The mouse residue matches the human consensus residue more than 50% of the time at 131 positions (102 positions match 90%-100% and 29 positions match 50% to 90%). These positions were not changed.

The lines labelled M/H in Figures 16A and 16B indicate the 91 positions which differed significantly between the mouse and human sequences (*i.e.*, where the human sequences have the mouse residue less than 50% of the time). Moderate risk positions, designated m in the M/H line, were kept "mouse"; whereas those designated H or h were changed to human. The 25 low risk positions which were already human-like or which were previously humanized (as described *supra* in Example 1) are designated " ^ " in the M/H line. Finally, the 54 high risk positions in which the mouse and human residues did not match are designated M and are kept "mouse".

Fifteen differences occur at moderate risk positions at which the mouse and human sequences differ. At ten of those positions (designated "H" on the M/H lines of Figure 6) the mouse residue aligns with a human consensus amino acid which is highly conserved. Therefore, the mouse residue at that position is identified as one to be changed to the conserved human residue.

At moderate risk positions (designated "m") in which the mouse and the human sequences differ, the mouse residue aligns with a human consensus amino acid which is

moderately conserved. However, since the mouse residue is found at that position in other actual sequences of human antibodies (in Kabat's sequences of Proteins of Immunoglobulin Interest, the positions are identified as ones to be kept "mouse." Although there are no such positions in this particular sequence, such positions may occur in other antibodies.

At four moderate risk positions (designated "h"), the mouse residue aligns with a human consensus amino acid which is moderately conserved but the mouse residue is not found at that position in an actual human antibody sequence in Kabat, *et al. Sequences of Proteins of Immunoglobulin Interest, supra.* Therefore, that position is identified as ones to be changed to "human."

At one moderate risk position (designated "m") in which the mouse and human sequences differ, the mouse residue aligns with a human consensus amino acid which is poorly conserved. Therefore, that position is identified as one to be kept "mouse."

The humanized H65 heavy chain containing the moderate risk residues was assembled by a strategy similar to that for the low risk residues. The moderate-risk expression vector was assembled from intermediate vectors. The six oligonucleotide sequences (oligos), disclosed in Figure 7B and labelled HUH-G11, HUH-G12, HUH-G3, HUH-G4, HUH-G5, and HUH-G6 (the sequences of HUH-G11 and HUH-G12 are set out in SEQ ID Nos. 56 and 57) were assembled by PCR. Oligonucleotides containing the synthetic humanized antibody gene were mixed in pairs (HUH-GI1 + HUH-G12, HUH-G3 + HUH-G4, and

HUH-G5 + HUH-G6) in a 100 μl reaction with 1 μg of each DNA and filled in as described above. A portion of each reaction product was mixed in pairs (HUH-G11, 12 + HUH-G3, 4; HUH-G3, 4 + HUH-G5, 6), 2.5 U Taq was added and samples were reincubated as described above. The in-J-region was assembled by mixing equal amounts of the HUH-Gil, 12, 3, 4 reaction product with the HUH-G3, 4, 5, 6 product, followed by PCR with 0.5 ug of primers H65G-2S and H65-G2 as described above. The reaction product was cut with SalI and BstEII and cloned into the expression vector, similar to that described for heavy chain in Robinson *et al.*, *Hum. Antibod. Hybridomas 2*:84 (1991), generating pING4617. That plasmid was sequenced with Sequenase (USB, Cleveland), revealing that two residues were altered (a G-A at position 288 and a A-T at position 312, numbered from the beginning of the leader sequence). The correct variable region was restored by substitution of this region from pING4612, generating the expected V-region sequence in pING4619.

An intermediate vector containing the other moderate-risk changes was constructed by PCR assembly of the oligos HUH-G13, HUH-G14, HUH-G15, and HUH-G16 (Fig. 7A and SEQ ID Nos: 58-61). Oligos HUH-G13 + HUH-G14 and HUH-G15 + HUH-G16 were mixed and filled in with Vent polymerase (New England Biotabs) in a reaction containing 10mM KCl, 20 mM TRIS pH 8.8, 10 mM $(NH_4)_2SO_2$, 2mM $MgSO_4$, 0.1% Triton X-100, 100 ng/ml BSA, 200 uM of each dNTP, and 2 units of Vent polymerase in a total volume of 100 μl. The reaction mix was incubated at 94°C for 1 minute, followed by 2 minutes at 50°C and 20 minutes at 72°C. The reaction products (40 μl) were mixed

and amplified with the oligonucleotides H65-G13 and H65-G2 with Vent polymerase in the same reaction buffer and amplified for 25 cycles with denaturation at 94°C for 1 minute, annealing at 50°C for 2 minutes and polymerization at 72°C for 3 minutes. The reaction product was treated with T4 polymerase and then digested with AccI. The 274 base pair (bp) fragment was purified on an agarose gel and ligated along with the 141 bp SalI to AccI fragment from pING4619 into pUC18 cut with SalI and SmaI to generate pING4620. pING4620 contains the entire signal sequence, V-region, and J-region of the moderate-risk H65 heavy chain.

The final expression vector for the moderate-risk H65 heavy chain, pING4621, was assembled by cloning the SalI to BstEII fragment from pING4620 into the same expression vector described above.

EXAMPLE 7

A. Assembly of moderate-risk light chain

The moderate-risk humanized V- and J-segments of the light chain were assembled from six oligonucleotides, $H65K-1, HUH-K7, HUH-K6, HUH-K8, HUH-K4 and HUH-K5. The sequences of HUH-K7, HUH-K6 and HUH-K8 are set out in SEQ ID Nos. 62-64 and Figs. 7 and 7A, respectively. The oligonucleotides were amplified with PCR primers H65K-2S and JK1-HindIII. Oligonucleotides containing the synthetic humanized antibody gene were mixed in pairs ($H65-K1 + HUH-K7, HUH-K6 + HUH-K4 + HUH-K5) and incubated with Vent polymerase as described for the moderate-risk heavy chain. A portion of each

reaction product (40 ul) was mixed in pairs ($H65H-K1/HUH-K7 + HUH-K6, 8; HUH-K6, 8 + HUH-K4, 5) and filled in as above. The light chain gene was then assembled by amplifying the full length gene with the PCR primers H65K-2S and JK1-HindIII with Vent polymerase for 25 cycles as outlined above. The assembled V/J region was cut with SalI and HindIII, purified by electrophoresis on an agarose gel, and assembled into a light chain antibody expression vector, pING4630.

B. Stable Transfection of Mouse Lymphoid Cells for the Production of He3 Antibody

The cell line Sp2/0 (American Type Culture Collection #CRL1581) was grown in Dulbecco's Modified Eagle Medium plus 4.5 g/l glucose (DMEM, Gibco) plus 10% fetal bovine serum. Media were supplemented with glutamine/penicillin/streptomycin (Irvine Scientific, Irvine, California).

The electroporation method of Potter, H., *et al.*, *Proc. Natl. Acad. Sci., USA*, 81:7161 (1984) was used. After transfection, cells were allowed to recover in complete DMEM for 24-48 hours, and then seeded at 10,000 to 50,000 cells per well in 96-well culture plates in the presence of selective medium. Histidinol (Sigma) selection was at 1.71 ug/ml, and mycophenolic acid (Calbiochem) was at 6 ug/ml plus 0.25 mg/ml xanthine (Sigma). The electroporation technique gave a transfection frequency of 1-10 x 10^{-5} for the Sp2/0 cells.

The He3 light chain expression plasmid pING4630 was linearized by digestion with PvuI restriction endonuclease and transfected into Sp2/0

cells, giving mycophenolic acid - resistant clones which were screened for light chain synthesis. The best 4 light chain - producing transfectants after outgrowth were pooled into 2 groups of 2 transfectants/pool and each pool was transfected with the He3 heavy chain expression plasmid, pING4621, that had been linearized with PvuI. After selection with histidinol, the clone producing the most light plus heavy chain, Sp2/0-4630 + 4621 Clone C1718, secreted antibody at approximately 22 μg/ul in the presence of 10^{-7} in dexamethasone in an overgrown culture in a T25 flask. This transfectoma has been deposited with the American Type Culture Collection, 1230 Parklawn Drive, Rockville, MD, 20852 on December 1, 1992 as ATCC HB 11206.

C. Purification of He3 Antibody Secreted in Tissue Culture

Sp2/0-4630 + 4621 cells are grown in culture medium HB101 (Hana Biologics) + 1% Fetal Bovine Serum, supplemented with 10mM HEPES, 1x Glutamine-Pen-Strep (Irvine Scientific #9316). The spent medium is centrifuged at about 5,000 x g for 20 minutes. The antibody level is measured by ELISA. Approximately 200ml of cell culture supernatant is loaded onto a 2ml Protein A-column (Sigma Chemicals), equiliberated with PBS (buffer 0.15 M NaCl, 5mM sodium phosphate, 1mM potassium phosphate, buffer pH 7.2). The He3 antibody is eluted with a step pH gradient (pH 5.5, 4.5 and 2.5). A fraction containing He3 antibody (9% yield) but not bovine antibody, is neutralized with 1 M Tris pH 8.5, and then concentrated 10-fold by Centrium 30 (Amicon) diluted

10-fold with PBS, reconcentrated 10-fold by Centricon 30, diluted 10-fold with PBS, and finally reconcentrated 10-fold. The antibody was stored in 0.25 ml aliquots at -20° C.

D. Affinity Measurements of He3 IgG for CD5

The affinity of He3 for CD5 was determined using Molt-4M cells, which express CD5 on their surface and I^{125}-labeled chimeric H65 IgG in a competitive binding assay.

For this assay, 20 μg of chimeric H65 IgG (cH65 IgG) was iodinated by exposure to 100 μl lactoperoxidase-glucose oxidase immobilized beads (Enzymobeads, BioRad), 100 μl of PBS, 1.0 mCi I^{125} (Amersham, IMS30), 50 μl of 55 mM b-D-glucose for 45 minutes at 23°C. The reaction was quenched by the addition of 20 μl of 105 mM sodium metabisulfite and 120 mM potassium iodine followed by centrifugation for 1 minute to pellet the beads. ^{125}I-cH65 IgG was purified by gel filtration using 7 mls of sephadex G25, using PBS (137 mM NaCl, 1.47 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 2.68 mM KCl at pH 7.2-7.4) plus 0.1% BSA. ^{125}I-cH65 IgG recovery and specific activity were determined by TCA precipitation.

Competitive binding was performed as follows: 100 μl of Molt-4M cells were washed two times in ice cold DHB binding buffer (Dubellco's modified Eagle's medium (Gibco, 320-1965PJ), 1.0% BSA and 10 mM Hepes at pH 7.2. -7.4). Cells were resuspended in the same buffer, plated into 96 v-bottomed wells (Costar) at 3 x 10^5 cells per well and pelleted at 4°C by centrifugation for 5 min at 1,000 rpm using a Beckman JS

4.2 rotor; 50 µl of 2X-concentrated 0.1 nM ^{125}I-cH65 IgG in DHB was then added to each well and competed with 50 µl of 2X - concentrated cH65 IgG or humanized antibody in DHB at final antibody concentrations from 100 nM to 0.0017 nM. Humanized antibody was obtained from culture supernatants of Sp2/0 clone C1718 which expresses He3 IgG. The concentration of the antibody in the supernatants was established by ELISA using a chimeric antibody as a standard. Binding was allowed to proceed at 4°C for 5 hrs and was terminated by washing cells three times with 200 µl of DHB binding buffer by centrifugation for 5 min at 1,000 rpm. All buffers and operations were at 4°C. Radioactivity was determined by solubilizing cells in 100 µl of 1.0 M NaOH and counting in a Cobra II auto gamma counter (Packard). Data from binding experiments were analyzed by the weighted nonlinear least squares curve fitting program, MacLigand, a Macintosh version of the computer program "Ligand" from Munson, *Analyt. Biochem.*, 107:220 (1980). Objective statistical criteria (F, test, extra sum squares principle) were used to evaluate goodness of fit and for discriminating between models. Nonspecific binding was treated as a parameter subject to error and was fitted simultaneously with other parameters.

The results of the competition binding assay are provided in Fig. 11. These results demonstrate that the moderate-risk changes made in He3 IgG result in an antibody with a higher affinity than the chimeric mouse-human form of this antibody (cH65) for it's targe, CD5. In this particular case, moderate risk changes appear to

increase affinity slightly, but a decrease may be expected in most cases.

EXAMPLE 8

Preparation of XMMLY-H65 Anti-pan T Cell Immunoglobulin

The murine monoclonal antibody produced by cell line XMMLY-H65 (MoAbH65) is reactive with the human CD5 antigen. The cell line XMMLY-H65 was deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, 20852 and designated Accession No. HB9286.

MoAb H65 was produced after immunization of BALB/c mice with the human T-cell line HSB-2 originally isolated from a patient with T-cell acute lymphocytic leukemia. Adams, *et al. Can. Res.* 28:1121 (1968). The murine myeloma cell line P3 7 NS/1-Ag-1-4 of Kohler *et al. Ernr. J. Immunol.* 6:292 (1976) was fused with spleen cells from an immunized mouse by the technique of Galfre *et al., Nature* 266:550 (1977). One of the resulting hybrid colonies was found to secrete a MoAb that recognizes a pan-T-lymphocyte antigen with a molecular weight of 67 kD, expressed on approximately 95% of peripheral T-lymphocytes [Knowles, *Leukocyte Typing II, 1,*(E. Reinherz, *et al.* eds., Springer Verlag (1986)]. This antigen is not present on the surface of any other hematopoietic cells, and the antibody itself has been tested for binding to a large range of normal human tissues and found to be negative for all cells except for T-lymphocytes and a subpopulation of B lymphocytes.

The H65 antibody-producing hybrid cell line was cloned twice by limiting dilution and was grown as ascites tumors in BALB/c mice.

MoAb H65 was purified from mouse ascites by a modification of the method of Ey *et al. Immunochem. 15*:429 (1978). In brief, the thawed mouse ascites was filtered to remove lipid-like materials and was diluted with 2 to 3 volumes of 0.14 M $NaPO_4$, pH 8.0, before application onto an immobilized protein A-Sepharose column of appropriate size. The unbound materials were removed from the column by washing with 0.14 M $NaPO_4$, pH 8.0, until no further change in absorbance at 280 nm was seen. A series of column washes with 0.1 M sodium citrate (pH 6.0, pH 5.0, pH 4.0, and pH 3.0) were then performed to elute bound antibody.

Peak fractions were pooled, adjusted to pH 7.0 with saturated Tris base, and concentrated by using a cell stirred with Amicon YM10 membrane (Amicon, Lexington, New York). An antibody solution was then dialyzed against phosphate-buffered saline (PBS), pH 7.0, and was stored frozen at -70°C.

MoAb H65 is of the IgG_1 subclass, as determined by double diffusion in agar with the use of subclass-specific antisera (Miles-Yeda, Ltd. Rehovot, Israel). The serologic characteristics of this antibody and the biochemical characteristics of the gp67 (i.e., CD5) antigen were examined during the First International Workshop on Human Leukocyte Differentiation Antigens (Paris, 1982). MoAb H65 (workshop number: T34), and nine other MoAbs were found to have the same serologic pattern and to immunoprecipiate the gp67 antigen. Knowles, *in*

Reinherz, *et al.*, *Leukocyte Typing II*, *2*: 259-288 (Springer-Verlag, 1986). In other studies, MoAb H65 has been shown to block the binding of FITC-conjugated anti-Leu-1 (Becton Dickson, Mountain View, CA) on gp67+ cells indicating that both antibodies recognize the same epitope on the gp67 molecule or determinants that are located in such a configuration as to result in blocking by steric hindrance.

EXAMPLE 9

The Use of Lyt-1 In The Prophylactic Treatment of CollagenInduced Arthritis in DBA/IJ mice

Collagen-induced arthritis (CIA) is a widely utilized model of human rheumatoid arthritis. CIA is characterized by a chronic polyarticular arthritis which can be induced in rodents and in primates by immunization with homologous or heterologous, native Type II collagen. The resulting arthritis resembles rheumatoid arthritis because there are similar histopathologic sequelae, cellular and humoral immune responses and restricted association with specific major histocompatibility complex (MHC) haplotypes.

Native, heterologous Type II collagen emulsified with complete Freund's adjuvant induces an arthritis-like autoimmune reaction in DBA/IJ mice after a single intradermal tail injection. The mice were obtained from Jackson Laboratories, Bar Harbor, Maine. Initially, the arthritis is noticeable as a slight swelling of one or more digits in the fourth week post-immunization. The chronic phase of CIA continually worsens over the ensuing 8 weeks as the arthritis

progresses from the digits into the remaining peripheral articulating joints and eventually ends with ankylosis of the involved joints. The histopathology of CIA is characterized by lymphocyte infiltration of the joint space, synovial MHC class II expression and pannus formation. Not all joints are involved on every mouse, so there is a spectrum of arthritic severity. In a group of ten or more mice, the overall arthritic severity develops in a linear fashion over the course of 10-12 weeks.

The CIA model was used to test the potential efficacy of a monoclonal antibody directed against the pan-T cell surface antigen, Lyt-1, the murine equivalent of CD5. The antibody was administered to the mice before the immunization with Type II collagen. Normal DBA/I mice were also treated with a single 0.4 mg/kg i.v. injection of anti-Lyt-1 and were sacrificed after 72 hours for FACS analysis and for *in vitro* proliferation assays on spleen and lymph node cells. Any efficacy of this antibody would indicate a beneficial T cell-directed approach in rheumatoid arthritis via the CD5 surface antigen.

Effects of anti-Lyt-1 on DBA/IJ Spleen Cells and Peripheral Lymph Nodes.

Antibody 53-7.313 is a rat IgG_{2a} monoclonal antibody (ATCC Accession No. TIB 104) reactive with all alleles of the mouse lymphocyte differentiation antigen, Lyt-1. The IND1 antibody is a mouse IgG_1, anti-human melanoma antibody used as a negative control (Xoma Corp., Berkeley, CA). All other antibodies were obtained from Pharmingen Inc. (San Diego, CA) as direct

conjugates for quantitation on a Becton-Dickinson FACScan instrument.

Male DBA/IJ mice, age 6-8 weeks, were administered a single intravenous dose of either phosphate buffered saline, IND1 or anti-Lyt-1 via the tail vein at 0.4 mg/kg in 0.1 ml of phosphate buffered saline. Mice were sacrificed for analysis three days after dosing. Single cell suspensions of spleens and peripheral lymph nodes were prepared by standard procedures and 1 x 10^6 cells were stained with the respective antibodies for fluorescence activated cell sorter (FACS) analysis. Proliferation assays were also performed to provide a second measure of T cell depletion. Cells (1 x 10^5/well) were stimulated with Concanavalin A, Interleukin-2 (IL-2), IL-2 and H57.597 (a pan α,β T cell receptor antibody) or the Staphylococcal enterotoxins A and B. Cells were cultured for a total of 72 hours and proliferation was quantitated by the addition of ^{3}H-methylthymidine for the last 24 hours. After 72 hours, the cells were harvested with an Inotech INB-384 harvesting and counting system, which collects the cells onto glass fiber filters with subsequent gas proportional beta particle detection. Results are generally expressed as the mean of triplicate wells ± SEM in Tables 5 and 6.

A. FACS Analysis of Lymph Node and Spleen Cells

FACS analysis of lymph node cells (LNC) and spleen cells (SPC) from each treatment group (n=3/group) were analyzed for percent expression of α,β T cell

receptor, CD3, CD4, CD5, and CD8. The results are presented in Table 4.

In Table 4, statistical significance was determined by Analysis of Variance followed by Duncan's New Multiple Range post-hoc test. These data indicate that administration of anti-Lyt-1 antibody results in a significant depletion of peripheral T lymphocytes at the 72 hour time point. The results could not be explained by residual circulating antibody as other T cell markers (CD3, etc.) are also depleted to a similar extent.

B. Effects of anti-Lyt-1 Administration on Proliferation Analysis

In vitro proliferation assays were performed on mice from each treatment group (n=3/group) in response to Concanavalin A, IL-2, IL-2 + H57, Staphylococcal enterotoxin A and B (SEA and SEB). The results are presented in Table 5.

Overall, these data indicate that there is an observable and functional depletion of DBA/IJ T peripheral lymphocytes 72 hours after a single (0.4 mg/kg) intravenous dose of anti-Lyt-1 antibody.

C. Effects of anti-Lyt-1 on Collagen-induced Arthritisin DBA/IJ Mice.

A. Materials and Methods

Male DBA/IJ mice, age 6-8 weeks, were administered the antibodies 53-7.313 (anti-Lyt-1), IND1 (anti-melanoma) or phosphate buffered saline (PBS) in two intravenous (0.4 mg/kg) doses 48 hours apart starting four days prior to immunization with 100 μg of bovine

type II collagen emulsified with an equal volume of Freund's complete adjuvant to a final injection volume of 100 μl. Each dose group was comprised of ten mice. Mice were monitored weekly starting on Day 21

TABLE 4. FACS Analysis of anti-Lyt-1 Treated DBA/1J Mice

TREAT-MENT	CELL TYPE	α, βTCR	CD3	CD4	CD8	CD5
PBS	LNC	80.2 ± 2.2%	79.8 ± 1.6%	58.7 ± 1.4%	19.4 ± 2.6%	80.0 ± 0.6%
IND1	LNC	82.5 ± 1.3%	82.6 ± 1.9%	60.9 ± 2.0%	21.1 ± 1.5%	78.5 ± 1.2%
αLyt-1	LNC	*62.7 ± 5.8%	*62.4 ± 1.0%	*42.0 ± 1.9%	21.1 ± 0.2%	*56.0 ± 2.6%
PBS	SPC	18.0 ± 2.8%	25.0 ± 0.1%	16.5 ± 2.1%	4.10 ± 0.5%	23.1 ± 0.1%
IND1	SPC	19.3 ± 1.6%	22.8 ± 1.4%	13.9 ± 0.8%	4.20 ± 0.3%	20.8 ± 1.5%
αLyt-1	SPC	14.0 ± 0.3%	*13.8 ± 0.4%	*8.07 ± 0.3%	*2.40 ± 0.1%	*11.0 ± 0.1%

TABLE 5. Proliferation Analysis of anti-Lyt-1 Treated DBA/1J mice.

TREAT-MENT	Concanavalin A	IL-2	IL-2 + H57	SEA	SEB
IND1	26547 ± 3501	1181 ± 234	11341 ± 1663	12324 ± 1968	8747 ± 2025
αLyt-1	*11561 ± 4375	*593 ± 274	*4090 ± 2383	*5568 ± 2576	*1138 ± 350

after immunization. Individual mice were scored for arthritic severity by grading each paw on a scale from 0 to 2. A score of 1 indicated swelling in up to two toes and a score of 2 indicated swelling in more than two toes up to total paw involvement and ankylosis of the large joint in the later time points. An individual mouse could have a maximum arthritic severity score of 8. Mice were monitored until day 80 after collagen immunization and then were sacrificed by cervical dislocation. Results are expressed as the mean arthritic score for each dose group.

The changes in arthritic score during the course of the study are shown in Figure 12. The overall conclusion in Figure 12 is that administration of the anti-Lyt-1 antibody prior to collagen immunization caused a significant decrease in the resulting severity of arthritis. In all of the treatment groups, the appearance of visible symptoms initiated at approximately 30 days after immunization and progressed linearly until the end of the study. The anti-Lyt-1 treatment group began to show ameliorated arthritic symptoms at Day 48 and never

developed arthritis to the same extent as the other two groups. The onset of arthritis was not significantly delayed by the anti-Lyt-1 treatment.

Statistical significance was determined by a Repeated Measures Analysis of Variance with one between subjects variable (antibody treatment). A Repeated Measures Analysis was necessary as each mouse was continually monitored for the duration of the study. Thus, the arthritic scores for consecutive days cannot be considered as independent observations contributing to the overall degrees of freedom in the F test for significant differences among groups. A Repeated Measures Analysis uses the degrees of freedom from the number of individuals per group instead of the number of observations. A typical between subjects Analysis of Variance may be inappropriate and may indicate false significance among the treatment groups. A comparison of means in the Treatment by Day after Immunization was done to determine the significance of anti-Lyt-1 treatment relative to PBS and IND1 control groups.

In conclusion, the intravenous administration of a rat monoclonal antibody reactive to the mouse equivalent of CD5, Lyt-1, is able to significantly decrease T lymphocytes in the spleen and in peripheral lymph nodes after a single 0.4 mg/kg dose. This T cell decrease is the probable mechanism for the significant ($p < 0.01$) decrease in arthritic

severity seen with the same anti-Lyt-1 dose prior to type II collagen immunization.

EXAMPLE 10

Depletion of Human T Cells From SCID Mice by Treatment With H65 MoAb

Severe combined immunodeficient (CB.17 *scid/scid*; SCID) mice maintain human lymphoid cells for several months following transplantation of human peripheral blood mononuclear cells (PBMC). Such chimeric mice, referred to as PBMC/SCID mice, have functional human cells, as shown by the presence of human Ig in their serum. PBMC/SCID mice maintain human T cells in tissues such as spleen and blood. Human T cells present in PBMC/SCID mice are predominantly of a mature phenotype and express T cell antigens, including CD3, CD5, CD7, and CD4 or CD8. In addition, most T cells appear to be activated memory cells, as judged by the expression of HLA-DR and CD45RO. These engrafted T cells appear to be functional since (a) they may provide help to B cells to produce anti-tetanus toxoid antibodies, (b) they produce soluble interleukin-2 receptor (sIL-2R) which may be detected in plasma, and (c) they proliferate in response to mitogenic anti-human CD3 monoclonal antibodies supplemented with IL-2 *in vitro*.

Because of the presence of human T and B cells, PBMC/SCID mice offer an *in vivo* model system in which to evaluate the efficacy of anti-human T cell drugs, such as H65 MoAb, a mouse IgGI directed against human CD5.

The SCID mice were obtained from Taconic, Germantown, NY, and at 6 to 7 weeks of age were injected with 200 mg/kg cyclophosphamide intraperitoneally (i.p.) to ensure engraftment of human PBMC. Two days later, 25 to 40 x 10^6 human PBMC, isolated by Ficoll-Hypaque density gradient centrifugation from lymphapheresis samples obtained from normal donors (HemaCare Corporation, Sherman Oaks, CA), were injected i.p.

At 2 to 3 weeks after PBMC injection, the mice were bled from the retro-orbital sinus and levels of human immunoglobulin (Ig) and human sIL-2R in plasma were quantified using sandwich ELISAs. Mice with low or undetectable levels of these human proteins were eliminated from the study and the remainder were divided into the various treatment groups (6 per group). The mice were then administered H65 MoAb (0.2 or 0.02 mg/kg/day), H65-based $F(ab')_2$ fragment (2 mg/kg/day) or vehicle (buffer) intravenously (i.v.) for 10 consecutive daily injections. One day after the last injection, the mice were bled and spleens were collected. Single cell suspensions of blood cells and splenocytes were prepared by standard methods. Recovered cells were then assayed for human T cell surface markers using flow cytometry.

Two to five hundred thousand cells were stained with the following FITC- or PE-conjugated Abs (Becton-Dickinson, Mountain View, CA): HLe-1-FITC (anti-CD45), Leu-2-FITC (anti-CD8), and Leu-3-PE (anti-CD4). Samples were analyzed on a FACScan using log amplifiers. Regions to quantify positive cells

were set based on staining of cells obtained from naive SCID mice. The absolute numbers of human antigen-positive cells recovered from SCID tissues were determined by multiplying the percent positive cells by the total number of cells recovered from each tissue sample. The total number of leukocytes in blood was calculated using a theoretical blood volume of 1.4 ml/mouse. Statistical comparisons between treatment groups were made using the Mann-Whitney U test.

The number of human T cells (CD4 plus CD8 cells) recovered from spleens and blood of PBMC/SCID mice following treatment with H65 MoAb or vehicle (control) is shown in Figure 13. Significantly lower numbers of T cells were recovered from spleens and blood of mice treated with either 0.2 or 0.02 mg/kg/day H65 MoAb as compared to vehicle-treated mice. In contrast, treatment with 2 mg/kg/day of an H65-based $F(ab')_2$ fragment did not significantly deplete human T cells from spleens or blood, even though a 10 to 100-fold higher dose was used (Figure 14).

These results indicate that an anti-human CD5 MoAb depletes human T cells in an experimental animal model. The ability of this MoAb to deplete human T cells from SCID mice is apparently dependent on the Fc portion of the MoAb, as an $F(ab')_2$ fragment was ineffective.

EXAMPLE 11

The Use of OX19 Monoclonal Antibody
In The Prophylactic Treatment of Collagen
Induced Arthritis in Diabetes-Resistant BB Rats

Collagen-induced arthritis (CIA) in the diabetes-resistant Biobreeding (DR BB) rat is a particularly relevant animal model of human rheumatoid arthritis, in that the DR BB rat RT1.Dβ gene encodes a nucleotide sequence homologous to the human HLA-DRβ gene reported to be associated with rheumatoid arthritis susceptibility. In this model, DR BB rats are administered a single intradermal tail injection of heterologous Type II collagen emulsified with incomplete Freund's adjuvant. Development of the arthritis is considerably faster than in the DBA/1J CIA model. Onset of clinical signs occurs 1.5 to 2 weeks after collagen immunization, with peak swelling observed a few days after onset. Incidence is generally quite high (>85% of animals immunized). The swelling is generally severe, involves the entire footpad and ankle joint, and is restricted to the hindlimbs. Histopathological examination has revealed that the arthritis begins as a proliferative synovitis with pannus formation at the joint margins that is followed by a bidirectional erosion of both the outer (unmineralized) and inner (mineralized) layers of cartilage.

This experiment uses the DR BB CIA rat model to assess the efficacy of a monoclonal antibody (MoAb), OX19 directed against the equivalent of the CD5 antigen in the rat. The antibody was administered to the rats

prior to immunization with Type II collagen. Normal Sprague-Dawley rats were also treated with a single 0.5 mg/kg i.v. injection and were sacrificed after 3 hours for evaluation of MoAb binding to T cells, or after 2 days for quantitation of T cells in lymphoid tissues using flow cytometry.

A. Effects of OX19 MoAb on T Cells In Lymphoid Tissues of Normal Sprague-Dawley Rats

OXI9 MoAb is a mouse IgG1 directed against the equivalent of rat CD5 antigen present on rat T cells. OX19 hybridoma is available from the European Collection of Animal Cell Cultures (ECACC) and has ECACC No. 84112012. H65 MoAb, a mouse IgG1 reactive against human CD5, was used as an isotype matched negative control. Fluorescein- conjugated antibodies directed against surface antigens on rat pan-T cells (W3/13), CD4 cells (W3/25) and CD8 cells (OX8) were obtained from Accurate Chemical and Scientific Corporation, Westbury, NY for flow cytometric quantitation of T cells in rat lymphoid tissues. Phycoerythrin-conjugated goat anti-mouse IgG1 (Caltag Laboratories, South San Francisco, CA) was used to detect OX19 MoAb bound to rat T cells in a two-color analysis.

Male Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA), 100 to 150 grams, were administered a single i.v. bolus injection of OX19 MoAb (0.5 mg/kg) or control MoAb (0.5 mg/kg) in phosphate buffered saline containing 0.1% Tween 80 (PBS/Tween). Animals were sacrificed at 3 hours (binding experiment)

or 2 days (depletion experiment) after dosing. Single cell suspensions of blood, spleens and lymph nodes were prepared by standard procedures and 1 x 10^6 cells were stained with appropriate antibodies for FACS analysis.

A. Binding of OX19 MoAb to Rat T Cells *In Vivo*.

Blood, spleen and lymph node cells from one animal in each treatment group were analyzed for percentage of CD4 and CD8 T cells, and percentage of CD4 and CD8 T cells that also stained positively for surface-bound mouse IgG1. The results are presented in Table 6. T cells were depleted from the blood at 3 hours after OX19 MoAb administration. Almost all of the T cells that remained in the blood, and most of those present in the spleen and lymph nodes in the OX19 MoAb-treated rat also stained positively for surface-bound mouse IgG1, indicating that the dose of OX19 MoAb used was sufficient to saturate most of the T cells in these major lymphoid organs. These results provide doses useful in therapeutic applications.

B. Effect of OX19 MoAb Treatment on T Cell Subpopulations in Rat Lymphoid Tissues.

Blood, spleen and lymph node cells from two animals in each treatment group were analyzed for percentage of pan-T, CD4 and CD8 cells. The results are presented in Table 7 as the mean of the two animals. OX19 MoAb treatment resulted in depletion of T cells from all tissues examined as compared to treatment with the control MoAb. These results also

provide appropriate doses to be used in therapeutic applications using antibodies according to the invention.

EXAMPLE 12

Effect of OX19 MoAb Treatment on Development of Collagen-Induced Arthritis in DR BB Rats

Male DR BB/Wor rats (obtained from the University of Massachusetts breeding facility; 8 per treatment group), age 6 weeks, were administered i.v. injections of OX19 MoAb (0.5 mg/kg), control MoAb (0.5 mg/kg) or buffer (PBS/Tween) on day 7 and day 4 prior to immunization at the base of the tail on day 0 with 0.3 mg of bovine Type II collagen emulsified in 0.15 ml

Table 6. Bind of OX19 MoAb to Rat T Cells *In Vivo*.

		% Positive Cell			
Tissue	Treatment	CD4	CD4/mIgG1*	CD8	CD8/mIgG1*
Blood	H65 MoAb	47.0	6.7	11.1	5.7
	OX19	8.7	96.2	4.1	70.2
Spleen	H65 MoAb	23.1	14.8	4.4	20.6
	OX19 MoAb	16.4	84.8	3.4	73.6
Lymph Node	H65 MoAb	66.9	4.2	7.4	6.5
	OX19 MoAb	54.7	96.2	7.3	96.8

* The % of CD4 or CD8 cells that are also positive for mouse IgG1.

Table 7. FACS Analysis of Tissues from OX19 MAb-Treated Rats.

		% Positive Cells		
Tissue	Treatment	Pan-T	CD4	CD8
Blood	H65 MoAB	61.8	50.4	12.0
	OX19 MoAb	47.0	37.3	8.8
Spleen	H65 MoAb	36.0	25.3	7.1
	OX19 MoAb	21.5	9.9	5.0
Lymph Node	H65 MoAb	74.5	62.7	13.1
	OX19 MoAb	33.8	24.9	4.3

of incomplete Freund's adjuvant. Rats were scored daily for arthritis beginning 8 days after collagen immunization. Severity was graded on a scale from 0 to 2, with a score of 1 indicating moderate swelling and a score of 2 indicating severe swelling. An individual animal could have a maximum arthritic severity score of 4 if there was bilateral hindlimb involvement.

The changes in arthritic score during the course of the study are shown in Figure 15 and the arthritic incidence for each treatment group is presented in Table 8.

Control (buffer and control MoAb-treated) rats developed severe, predominantly bilateral hindlimb arthritis between days 10 and 14 with high incidence (88% for both groups). Treatment with OX19 MoAb completely prevented development of arthritis (0% incidence).

In conclusion, a 0.5 mg/kg intravenous dose of a mouse MoAb directed against the rat equivalent of CD5 was found to saturate and subsequently deplete T cells from lymphoid tissues of normal rats. This T cell depletion is the probable mechanism for the complete inhibition of arthritis development observed when the MoAb was administered prior to Type II collagen immunization in DR BB rats.

EXAMPLE 13

Treatment of Rheumatoid Arthritis

Patients having rheumatoid arthritis (RA) are selected for treatment using an anti-pan T cell antibody of this invention.

Anti-CD5 antibody prepared as described above is administered to patients at doses of about 0.005 to

2.0 mg/kg/day for a period of 1-5 days, preferably 1-2 days. Alternatively, the dose may be given every 2-30

Table 8. Effect of OX19 MoAb Treatment on Arthritis Incidence

TREATMENT	Total arthritics (1 or both limbs)	Total Arthritics (Both limbs)	Score of "2" (1 or both limbs)	Score of "2" (Both Limbs)
PBS/Tween	7/8 (88%)	7/8 (88%)	7/8 (88%)	5/8 (63%)
Control MoAb	7/8 (88%)	4/8 (50%)	6/8 (75%)	4/8 (50%)
OX19 MoAb	0/8 (0%)	0/8 (0%)	0/8 (0%)	0/8 (0%)

days instead of daily if chimeric and humanized MoAbs are used due to their increased half-life. To determine optimum dose and schedule, patients are treated at each dose and schedule in a dose escalating regimen. Patients are monitored using several indicia, including joint swelling and tenderness scores. Results are shown in Figure 11.

EXAMPLE 14

Treatment of SLE

Systemic Lupus Erythematosus (SLE) is a multisystemic disease characterized by inflammation and autoimmunity. Some of the more frequent manifestations include fatigue, anemia, fever, rashes, photosensitivity, alopecia, arthritis, pericarditis, pleurisy, vasculitis, nephritis and central nervous system disease. Under the Revised Criteria for Classification of SLE, a person is said to have SLE for purposes of clinical studies if any four or more of the aforementioned specified criteria are present, serially or simultaneously, during any interval of observation.

Anti-CD5 antibody prepared as described above is administered to patients at doses of about 0.005 to 2.0 mg/kg/day for a period of 1-5 days, preferably 1-2 days. Alternatively, the dose may be given every 2-30 days instead of daily if chimeric and humanized MoAbs are used due to their increased half-life. To determine optimum dose and schedule, patients are treated at each dose and schedule in a dose escalating regimen.

EXAMPLE 15

Treatment of Psoriasis

Psoriasis is a disease of autoimmune etiology which Classically appears as plaques over the elbows and

knees, although other areas of the skin are frequently afflicted. Abnormalities of the nails and the joints are also frequently observed. Particularly inflammatory joint disease can occur in an occasionally erosive and severe form.

Anti-CD5 antibody prepared as described above is administered to patients at doses of about 0.005 to 2.0 mg/kg/day for a period of 1-5 days, preferably 1-2 days. Alternatively, the dose may be given every 2-30 days instead of daily if chimeric and humanized MoAbs are used due to their increased half-life. To determine optimum dose and schedule, patients are treated at each dose and schedule in a dose escalating regimen.

Clinical observation includes evaluation of the patient's overall status as well as special attention to the psoriatic plaques. Additionally, monitoring of laboratory parameters such as white blood count and differential are recommended. Symptoms which may indicate poor tolerance to therapy or complications include nausea, vomiting, fatigue, rash, fever, chills and syncope. Any unexplained depletion in white blood cells other than lymphocytes is an indication to discontinue therapy. Preferably, differential analysis of lymphocytes is carried out. That is, analysis of the total number of T cells and B cells should be determined.

EXAMPLE 16

Treatment of Type I Diabetes

There are two major types of diabetes. Type I has classically been associated with a requirement for exogenous insulin. Type I typically occurs before the age of 40 and is associated with an absence of insulin secretion. The pancreas of pati nts with long-term Type

I insulin-dependent diabetes are devoid of pancreatic islet cells. There is a large body of evidence that the etiology of Type I insulin-dependent diabetes (IDDM) is autoimmune.

Patients are diagnosed as having IDDM based on the criteria established by the American Diabetes Association. Anti-CD5 antibody prepared as described above is administered to patients at doses of about 0.005 to 2.0 mg/kg/day for a period of 1-5 days, preferably 1-2 days. Alternatively, the dose may be given every 2-30 days instead of daily if chimeric and humanized MoAbs are used due to their increased half-life. To determine optimum dose and schedule, patients are treated at each dose and schedule in a dose escalating regimen.

During the study, the patients were monitored by clinical and laboratory parameters. Clinical symptoms indicating poor tolerance to therapy or complications include fatigue, vomiting, rash, fever, chills, and syncope. Laboratory evaluation included white blood cell counts with differential analysis daily and blood glucose levels at least twice a day.

Using diagnostic criteria predictive of the onset of Type I diabetes, patients may be selected for prophylactic treatment. This treatment follows the dose and schedule noted above for treatment of clinical insulin dependent diabetes.

While the invention has been described in terms of specific examples and preferred embodiments, is understood that variations and improvements will occur to those skilled in the art. Therefore, it is recognized that there are numerous variations and improvements which come within the scope of the invention as claimed.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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Little II, Roger G.
Fishwild, Dianne M.
Kohn, Fred R.

(ii) TITLE OF INVENTION: Methods and Materials for Preparation of Modified Antibody Variable Domains and Theraputic Uses Thereof

(iii) NUMBER OF SEQUENCES: 64

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(B) REGISTRATION NUMBER: 30,060
(C) REFERENCE/DOCKET NUMBER: 27129/30637

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(C) TELEX: 25-3856

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Asp Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Val Thr Pro Gly
1 5 10 15

Asn Ser Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Gly Asn Asn
20 25 30

Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Leu Leu Ile
35 40 45

Lys Tyr Ala Ser Gln Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Thr
65 70 75 80

Glu Asp Phe Gly Met Tyr Phe Cys Gln Gln Ser Asn Ser Trp Pro Tyr
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Asp Ile Lys
100 105

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 113 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ser Val Ser Ala Gly
1               5                  10                  15

Glu Arg Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser
            20                  25                  30

Gly Asn Gln Lys Asn Phe Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
        35                  40                  45

Pro Pro Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val
    50                  55                  60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65                  70                  75                  80

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn
                85                  90                  95

Asp His Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Ile
            100                 105                 110

Lys
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 103 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Xaa Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
20 25 30

Asn His Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45

Leu Ile Phe His Asn Asn Ala Arg Phe Ser Val Ser Lys Ser Gly Ser
50 55 60

Ser Ala Thr Leu Ala Ile Thr Gly Leu Gln Ala Glu Asp Glu Ala Asp
65 70 75 80

Tyr Tyr Cys Gln Ser Tyr Asp Arg Ser Leu Arg Val Phe Gly Gly Gly
85 90 95

Thr Lys Leu Thr Val Leu Arg
100

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 111 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Gln Ser Val Leu Thr ln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln

```
1               5                   10                  15

Arg Val Thr Ile Ser Cys Ser Gly Thr Ser Ser Asn Ile Gly Ser Ser
            20                  25                  30

Thr Val Asn Trp Tyr Gln Gln Leu Pro Gly Met Ala Pro Lys Leu Leu
        35                  40                  45

Ile Tyr Arg Asp Ala Met Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50                  55                  60

Gly Ser Lys Ser Gly Ala Ser Ala Ser Leu Ala Ile Gly Gly Leu Gln
65                  70                  75                  80

Ser Glu Asp Glu Thr Asp Tyr Tyr Cys Ala Ala Trp Asp Val Ser Leu
                85                  90                  95

Asn Ala Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
            100                 105                 110
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 113 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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Asp Val Gln Leu Gln Glu Ser Gly Pro Ser Leu Val Lys Pro Ser Gln
1               5                   10                  15

Thr Leu Ser Leu Thr Cys Ser Val Thr Gly Asp Ser Ile Thr Ser Asp
            20                  25                  30

Tyr Trp Ser Trp Ile Arg Lys Phe Pro Gly Asn Arg Leu Glu Tyr Met
        35                  40                  45
```

Gly Tyr Val Ser Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser Leu Lys
50 55 60

Ser Arg Ile Ser Ile Thr Arg Asp Thr Ser Lys Asn Gln Tyr Tyr Leu
65 70 75 80

Asp Leu Asn Ser Val Thr Thr Glu Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Asn Trp Asp Gly Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser
100 105 110

Ala

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 122 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Phe Thr Phe Ser Asp Phe
20 25 30

Tyr Met Glu Trp Val Arg Gln Pro Pro Gly Lys Arg Leu Glu Trp Ile
35 40 45

Ala Ala Ser Arg Asn Lys Gly Asn Lys Tyr Thr Thr Glu Tyr Ser Ala
50 55 60

Ser Val Lys Gly Arg Phe Ile Val Ser Arg Asp Thr Ser Gln Ser Ile

65 70 75 80

Leu Tyr Leu Gln Met Asn Ala Leu Arg Ala Glu Asp Thr Ala Ile Tyr
85 90 95

Tyr Cys Ala Arg Asn Tyr Tyr Gly Ser Thr Trp Tyr Phe Asp Val Trp
100 105 110

Gly Ala Gly Thr Thr Val Thr Val Ser Ser
115 120

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gln Val Gln Leu Glu Gln Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Thr Ser Phe Asp Asp Tyr
20 25 30

Tyr Ser Thr Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Val Phe Tyr His Gly Thr Ser Asp Thr Asp Thr Pro Leu Arg
50 55 60

Ser Arg Val Thr Met Leu Val Asn Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80

Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Asn Leu Ile Ala Gly Cys Ile Asp Val Trp Gly Gln Gly Ser Leu
100 105 110

Val Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 126 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Glu Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ser Ser Ser Gly Phe Ile Phe Ser Ser Tyr
20 25 30

Ala Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Ile Ile Trp Asp Asp Gly Ser Asp Gln His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asn Asp Ser Lys Asn Thr Leu Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Asp Gly Gly His Gly Phe Cys Ser Ser Ala Ser Cys Phe Gly
100 105 110

Pro Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser

115 120 125

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Asn Ser Gly Asn Gln Lys
1 5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Asn Lys Gly
1

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gly Ser Thr
1

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

His Gly Phe Cys Ser Ser Ala Ser Cys
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Xaa Ile Ser Xaa Tyr
20 25 30

Leu Xaa Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Xaa Leu Xaa Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Xaa Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Xaa Xaa Xaa Pro Xaa
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr ly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser Gly

50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro
65 70 75 80

Gly Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro Xaa
85 90 95

Thr Phe Gly Gln Gly Thr Asp Val Glu Ile Lys
100 105

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu Asn Asn
20 25 30

Tyr Leu Asn Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu
35 40 45

Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu
65 70 75 80

Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Xaa Pro
85 90 95

Xaa Thr Phe Gly Gln Gly Thr Lys Xaa Glu Ile Lys
100 105

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 106 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Xaa Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Ile Gly Xaa Asn Xaa
20 25 30

Val Xaa Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Asp Leu Leu Ile
35 40 45

Tyr Asn Asn Arg Pro Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Lys
50 55 60

Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln Ser Glu Asp
65 70 75 80

Glu Ala Asp Tyr Tyr Cys Ala Thr Trp Asp Asp Ser Leu Asp Pro Val
85 90 95

Phe Gly Gly Gly Thr Lys Thr Val Leu Gly
100 105

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 104 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Xaa Ser Ala Leu Thr Gln Pro Ala Ser Val Ser Gly Ser Pro Gly Gln
1 5 10 15

Ser Ile Thr Ile Ser Cys Thr Gly Thr Ser Ser Val Gly Tyr Asn Xaa
20 25 30

Val Ser Trp Tyr Gln Gln His Pro Gly Lys Ala Pro Lys Leu Ile Tyr
35 40 45

Asp Val Arg Pro Ser Gly Val Arg Phe Ser Gly Ser Lys Ser Gly Asn
50 55 60

Thr Ala Ser Leu Thr Ile Ser Gly Leu Gln Ala Glu Asp Glu Ala Asp
65 70 75 80

Tyr Tyr Cys Ser Ser Tyr Xaa Gly Xaa Xaa Xaa Xaa Val Phe Gly Gly
85 90 95

Gly Thr Lys Leu Thr Val Leu Gly
100

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 100 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Ser Tyr Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ser Pro Gly Gln
1 5 10 15

Thr Ala Ile Thr Cys Ser Gly Asp Xaa Leu Xaa Xaa Xaa Tyr Val Xaa
20 25 30

Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr Asp
35 40 45

Arg Pro Ser Gly Ile Pro Gln Arg Phe Ser Gly Ser Ser Thr Thr Ala
50 55 60

Thr Leu Thr Ile Ser Gly Val Gln Ala Asp Glu Ala Asp Tyr Tyr Cys
65 70 75 80

Gln Xaa Trp Asp Xaa Xaa Xaa Val Val Phe Gly Gly Gly Thr Lys Leu
85 90 95

Thr Val Leu Gly
100

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 106 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Asn Phe Met Leu Thr Gln Pro His Ser Val Ser Glu Ser Pro Gly Lys
1 5 10 15

Thr Val Thr Ile Ser Cys Thr Xaa Ser Xaa Gly Ile Ala Ser Xaa Tyr
20 25 30

Val Gln Trp Tyr Gln Gln Arg Pro Gly Ser Ala Pro Thr Thr Val Ile
35 40 45

Tyr Glu Asp Asn Arg Pro Ser Gly Val Pro Asp Arg Phe Ser Gly Ser
50 55 60

Ser Ser Asn Ser Ala Ser Leu Thr Ile Ser Gly Leu Lys Thr Glu Asp
65 70 75 80

Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Xaa Xaa Trp Val Phe
85 90 95

Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Lys Asn
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu
35 40 45

Ile Tyr Trp Ala Ser Arg Glu Ser Gly Val Pro Asp Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala

65 70 75 80

Gln Asp Val Ala Val Tyr Tyr Cys Gln Gln Tyr Tyr Ser Thr Pro Xaa
85 90 95

Thr Phe Gly Gly Gly Thr Lys Xaa Gly Ile Lys
100 105

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 105 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Ser Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln Thr
1 5 10 15

Arg Ile Thr Cys Ser Gly Asp Xaa Leu Gly Xaa Tyr Asp Ala Xaa Trp
20 25 30

Tyr Gln Gln Lys Pro Gly Gln Ala Pro Leu Leu Val Ile Tyr Gly Arg
35 40 45

Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser Ser Ser Gly
50 55 60

His Thr Ala Ser Leu Thr Ile Thr Gly Ala Gln Ala Glu Asp Glu Ala
65 70 75 80

Asp Tyr Tyr Cys Asn Ser Arg Asp Ser Ser Gly Lys Val Leu Phe Gly
85 90 95

Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 96 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
Ser Ala Leu Thr Gln Pro Pro Ser Ala Ser Gly Ser Pro Gly Gln Ser
1               5                  10                  15

Val Thr Ile Ser Cys Thr Gly Thr Ser Ser Val Gly Xaa Xaa Tyr Val
            20                  25                  30

Ser Trp Tyr Gln Gln His Gly Ala Pro Lys Ile Glu Val Arg Pro Ser
        35                  40                  45

Gly Val Pro Asp Arg Phe Ser Gly Ser Lys Ser Asn Thr Ala Ser Leu
    50                  55                  60

Thr Val Ser Gly Leu Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ser Ser
65                  70                  75                  80

Tyr Xaa Xaa Xaa Xaa Xaa Phe Val Phe Gly Gly Thr Lys Thr Val Leu
                85                  90                  95
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Xaa Xaa
20 25 30

Xaa Met Xaa Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Xaa Xaa Ile Xaa Xaa Lys Xaa Xaa Gly Xaa Xaa Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

ln Val ln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Xaa

1 5 10 15

Ser Val Xaa Val Ser Cys Lys Xaa Ser Gly Tyr Tyr Phe Xaa Xaa Tyr
20 25 30

Xaa Ile Xaa Trp Val Arg Gln Ala Pro Gly Xaa Gly Leu Glu Trp Val
35 40 45

Gly Xaa Ile Xaa Pro Xaa Xaa Gly Xaa Thr Xaa Tyr Ala Pro Xaa Phe
50 55 60

Gln Gly Arg Val Thr Xaa Thr Arg Asp Xaa Ser Xaa Asn Thr Ala Tyr
65 70 75 80

Met Glu Leu Xaa Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Xaa Val Thr Leu Xaa Glu Ser Gly Pro Xaa Leu Val Leu Pro Thr Gln
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Xaa Ser Leu Ser Xaa Xaa
20 25 30

Xaa Val Xaa Trp Ile Arg Gln Pro Pro Gly Lys Xaa Leu Glu Trp Leu
35 40 45

Ala Xaa Ile Xaa Ile Asp Asp Asp Xaa Tyr Xaa Thr Ser Leu Arg Ser
50 55 60

Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val Val Leu Xaa
65 70 75 80

Xaa Xaa Xaa Xaa Asp Pro Xaa Asp Thr Ala Thr Tyr Tyr Cys Ala Arg
85 90 95

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Val Trp Gly Gln Gly Thr Thr
100 105 110

Val Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met Tyr Ala Ser Leu Gly
1 5 10 15

Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Asn Ser Tyr
20 25 30

Leu Ser Trp Phe Gln Gln Lys Pro Gly Lys Ser Pro Lys Thr Leu Ile
35 40 45

Tyr Arg Ala Asn Arg Leu Val Asp Gly Val Pro Ser Arg Phe Ser Gly

50 55 60

Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile Ser Ser Leu Asp Tyr
65 70 75 80

Glu Asp Met Gly Ile Tyr Tyr Cys Gln Gln Tyr Asp Glu Ser Pro Trp
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Met Ser Ala Ser Leu Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Asn Ser Tyr
20 25 30

Leu Ser Trp Phe Gln Gln Lys Pro Gly Lys Ser Pro Lys Thr Leu Ile
35 40 45

Tyr Arg Ala Asn Arg Leu Val Asp Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Tyr
65 70 75 80

Glu Asp Phe Gly Ile Tyr Tyr Cys Gln Gln Tyr Asp Glu Ser Pro Trp
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 118 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
1 5 10 15

Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Arg Trp Met
35 40 45

Gly Trp Ile Asn Thr His Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys
85 90 95

Thr Arg Arg Gly Tyr Asp Trp Tyr Phe Asp Val Trp Gly Ala Gly Thr
100 105 110

Thr Val Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 118 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
Gln Ile Gln Leu Val Gln Ser Gly Pro Gly Leu Lys Lys Pro Gly Gly
1               5                  10                  15

Ser Val Arg Ile Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30

Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Arg Trp Met
        35                  40                  45

Gly Trp Ile Asn Thr His Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe
    50                  55                  60

Lys Gly Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Ile Asn Ser Leu Arg Ala Glu Asp Thr Ala Thr Tyr Phe Cys
                85                  90                  95

Thr Arg Arg Gly Tyr Asp Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr
            100                 105                 110

Thr Val Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

AGTCGTCGAC ACGATGGACA TGAGGACCCC TGCTCAGTTT CTTGGCATCC TCCTACTCTG 60

GTTTCCAGGT ATCAAATGTG ACATCCAGAT GACTCAGT 98

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 80 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TGACTCGCCC GGCAAGTGAT AGTGACTCTG TCTCCCAGAC ATGCAGACAT GGAAGATGAG 60

GACTGAGTCA TCTGGATGTC 80

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 79 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TCACTTGCCG GGCGAGTCAG GACATTAATA GCTATTTAAG CTGGTTCCAG CAGAAACCAG 60

GGAAATCTCC TAAGACCCT 79

(2) *INFORMATION FOR SEQ ID NO:33:*

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 79 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GATCCACTGC CACTGAACCT TGATGGGACC CCATCTACCA ATCTGTTTGC ACGATAGATC 60

AGGGTCTTAG GAGATTTCC 79

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 82 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GGTTCAGTGG CAGTGGATCT GGGACAGATT ATACTCTCAC CATCAGCAGC CTGCAATATG 60

AAGATTTTGG AATTTATTAT TG 82

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 82 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GTTTGATTTC AAGCTTGGTG CCTCCACCGA ACGTCCACGG AGACTCATCA TACTGTTGAC 60

AATAATAAAT TCCAAAATCT TC 82

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

TGTCGACATC ATGGCTTGGG TGTGGACCTT GCTATTCCTG ATGGCAGCTG CCCAAAGTGC 60

CCAAGCACAG ATCCAGTTGG TGCAG 85

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

AAGGTATACC CAGAAGCTGC GCAGGAGATT CTGACGGACC CTCCAGGCTT CTTCAGGCCA 60

GGTCCAGACT GCACCAACTG GATCT 85

(2) *INFORMATION FOR SEQ ID NO:38:*

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GCAGCTTCTG GGTATACCTT CACAAACTAT GGAATGAACT GGGTGAAGCA GGCTCCAGGA 60

AAGGGTTTAA GGTGGATGGG CTGG 84

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

AAAGAGAAGG TAAACCGTCC CTTGAAGTCA TCAGCATATG TTGGCTCTCC AGTGTGGGTG 60

TTTATCCAGC CCATCCACCT TAAAC 85

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GACGGTTTAC CTTCTCTTTG GACACGTCTA AGTGCACTGC CTATTTACAG ATCAACAGCC 60

TCAGAGCCGA GGACACGGCT ACAT 84

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 91 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

AGGAGACGGT GACCGTGGTC CCTTGGCCCC AGACATCGAA GTACCAGTCG TAACCCCGTC 60

TTGTACAGAA ATATGTAGCC GTGTCCTCGG C 91

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

ACTAGTGTCG ACATCATGGC TTGGGT 26

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GAGGAGACGG TGACCGTGGT 20

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

AGTCGTCGAC ACGATGGACA TGAGGAC 27

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

GTTTGATTTC AAGCTTGGTG C 21

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 425 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

ACTAGTGTCG ACATCATGGC TTGGGTGTGG ACCTTGCTAT TCCTGATGGC AGCTGCCCAA 60

AGTGCCCAAG CACAGATCCA GTTGGTGCAG TCTGGACCTG GCCTGAAGAA GCCTGGAGGG 120

TCCGTCAGAA TCTCCTGCGC AGCTTCTGGG TATACCTTCA CAAACTATGG AATGAACTGG 180

GTGAAGCAGG CTCCAGGAAA GGGTTTAAGG TGGATGGGCT GGATAAACAC CCACACTGGA 240

GAGCCAACAT ATGCTGATGA CTTCAAGGGA CGGTTTACCT TCTCTTTGGA CACGTCTAAG 300

AGCACTGCCT ATTTACAGAT CAACAGCCTC AGAGCCGAGG ACACGGCTAC ATATTTCTGT 360

ACAAGACGGG GTTACGACTG GTACTTCGAT GTCTGGGGCC AAGGGACCAC GGTCACCGTC 420

TCCTC 425

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 401 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

AGTCGTCGAC ACGATGGACA TGAGGACCCC TGCTCAGTTT CTTGGCATCC TCCTACTCTG 60

GTTTCCAGGT ATCAAATGTG ACATCCAGAT GACTCAGTCT CCATCTTCCA TGTCTGCATC 120

TCTGGGAGAC AGAGTCACTA TCACTTGCCG GGCGAGTCAG GACATTAATA GCTATTTAAG 180

CTGGTTCCAG CAGAAACCAG GGAAATCTCC TAAGACCCTG ATCTATCGTG CAAACAGATT 240

GGTAGATGGG GTCCCATCAA GGTTCAGTGG CAGTGGATCT GGGACAGATT ATACTCTCAC 300

CATCAGCAGC CTGCAATATG AAGATTTTGG AATTTATTAT TGTCAACAGT ATGATGAGTC 360

TCCGTGGACG TTCGGTGGAG GCACCAAGCT TGAAATCAAA C 401

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Asn Thr Trp
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Met
        35                  40                  45

Tyr Lys Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ile Gly
    50                  55                  60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Asp Ser Lys
                85                  90                  95

Met Phe Gly Gln Gly Thr Lys Val Glu Val Lys
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 106 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1 5 10 15

Glu Lys Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Ile Ser Tyr Met
20 25 30

His Trp Phe Gln Gln Lys Pro Gly Thr Ser Pro Lys Leu Trp Ile Tyr
35 40 45

Thr Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Met Glu Ala Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys His Gln Arg Ser Thr Tyr Pro Leu Thr
85 90 95

Phe Gly Ser Gly Thr Lys Leu Glu Leu Lys
100 105

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 106 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Asp Ile Gln Leu Thr ln Ser Pro Ser Ser Met Ser Ala Ser Pro Gly

```
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Ile Ser Tyr Met
            20                  25                  30

His Trp Phe Gln Gln Lys Pro Gly Lys Ser Pro Lys Leu Trp Ile Tyr
        35                  40                  45

Thr Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Ser Tyr Thr Leu Thr Ile Ser Ser Met Gln Ala Glu
65                  70                  75                  80

Asp Phe Ala Thr Tyr Tyr Cys His Gln Arg Ser Thr Tyr Pro Leu Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Glu Leu Lys
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 106 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Ile Ser Tyr Met
            20                  25                  30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
        35                  40                  45
```

```
Thr Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65                  70                  75                  80

Asp Phe Ala Thr Tyr Tyr Cys His Gln Arg Ser Thr Tyr Pro Leu Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Val Glu Val Lys
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Arg Ser
            20                  25                  30

Ala Ile Ile Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Gly Ile Val Pro Met Phe Gly Pro Pro Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Asn Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Phe Tyr Phe Cys
```

85 90 95

Ala Gly Gly Tyr Gly Ile Tyr Ser Pro Glu Glu Tyr Asn Gly Gly Leu
100 105 110

Val Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 116 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Gln Val Gln Leu Gln Gln Ser Phe Ala Glu Leu Ala Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Arg Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Thr Gly Tyr Thr Glu Tyr Asn Gln Lys Phe
50 55 60

Lys Asp Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Phe Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Gly Gly Val Phe Asp Tyr Trp Gly Gln Gly Thr Thr Leu
100 105 110

Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 116 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Gln Val Gln Leu Gln Gln Ser Phe Ala Glu Val Ala Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Arg Met His Trp Val Lys Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Thr Gly Tyr Thr Glu Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Gly Gly Val Phe Asp Tyr Trp Gly Gln Gly Thr Thr Leu
100 105 110

Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 116 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

```
Gln Val Gln Leu Val Gln Ser Phe Ala Glu Val Lys Lys Pro Gly Ser
1               5                  10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30

Arg Met His Trp Val Lys Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Thr Gly Tyr Thr Glu Tyr Asn Gln Lys Phe
    50                  55                  60

Lys Asp Lys Ala Thr Ile Thr Ala Asp Glu Ser Thr Asn Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Gly Gly Gly Val Phe Asp Tyr Trp Gly Gln Gly Thr Thr Val
            100                 105                 110

Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

TGTCGACATC ATGGCTTGGG TGTGGACCTT GCTATTCCTG ATGGCAGCT GCCCAAAGTG 60
CCCAAGCAGAGATC CAGTTGGTGCA G 82

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

AAGGTATACC CAGAAGCTGC GCAGGAGATT CTGACGGACC CTCCAGGCTT CACCAGGCCT 60

CCTCCAGACT GCACCAACTG GATCTC 86

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

GCAGCTTCTG GGTATACCTT CACAAACTAT GGAATGAACT GGGTGCGCCA GGCTCCAGGA 60

AAGAATTTAG AGTGGATGGG CTGG 84

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

AAAGAGAAGG TAAACCGTCC CTTGAAAGAA TCAGCATATG TTGGCTCTCC AGTGTGGGTG 60

TTTATCCAGC CCATCCACTC TAAAC 85

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 87 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

GACGGTTTAC CTTCTCTTTG GACGATTCTA AGAACACTGC CTATTTACAG ATCAACAGCC 60

TCAGAGCCGA GGACACGGCT GTGTATT 87

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 92 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

GAGGAGACGG TGACCGTGGT CCCTTGGCCC CAGACATCGA AGTACCAGTC GTAACCCCGT 60

CTTGTACAGA AATACACAGC CGTGTCCTCG GC 92

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 80 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

TGACTCGCCC GGCAAGTGAT AGTGACTCTG TCTCCTACAG ATGCAGACAG GGAAGATGGA 60

GACTGAGTCA TCTGGATGTC 80

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

TCACTTGCCG GGCGAATCAG GACATTAATA GCTATTTAAG CTGGTTCCAG CAGAAACCAG 60

GGAAAGCTCC TAAGACCCT 76

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 79 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

GATCCACTGC CACTGAACCT TGATGGGACC CCAGATTCCA ATCTGTTTGC ACGATAGATC 60

AGGGTATTAG GAGCTTTCC 79

CLAIMS

1. A method for preparing a modified antibody variable domain useful for administration to humans, comprising the steps of:

(a) determining the amino acid sequence of a subject light chain and a subject heavy chain of a subject antibody variable domain to be modified;

(b) aligning by homology: the subject light chain amino acid sequence with a plurality of human light chain amino acid sequences; and the subject heavy chain amino acid sequences with a plurality of human heavy chain amino acid sequences;

(c) identifying the amino acids in the subject light and heavy chain sequences which are least likely to diminish the native affinity of the subject variable domain for antigen while, at the same time, reducing its immunogenicity by selecting each amino acid which is not in an interface region of the subject antibody variable domain and which is not in a complementarity-determining region or in an antigen-binding region of the subject antibody variable domain, but which amino acid is in a position exposed to a solvent containing the antibody; and

(d) changing each amino acid identified in (c) which aligns with a highly or moderately conserved amino acid in the plurality of human heavy or light chain amino acid

sequences if said identified amino acid is different from the amino acid in the plurality of human heavy or light chain amino acid sequences.

2. The method as recited in claim 1, wherein said plurality of human light chain amino acid sequences is selected from the group consisting of:

human kappa light chain subgroup 1 consensus sequence as set out in SEQ ID NO: 9 and in Figure 5A;

human kappa light chain subgroup 3 consensus sequence as set out in SEQ ID NO: 10 and in Figure 5A;

human kappa light chain subgroup 2 consensus sequence as set out in SEQ ID NO: 11 and in Figure 5A;

human lambda light chain subgroup 1 consensus sequence as set out in SEQ ID NO: 12 and in Figure 5A;

human lambda light chain subgroup 2 consensus sequence as set out in SEQ ID NO: 13 and in Figure 5A;

human lambda light chain subgroup 3 consensus sequence as set out in SEQ ID NO: 14 and in Figure 5A;

human lambda light chain subgroup 6 consensus sequence as set out in SEQ ID NO: 15 and in Figure 5A;

human kappa light chain subgroup 4 consensus sequence as set out in SEQ ID NO: 16 and in Figure 5A;

human lambda light chain subgroup 4 consensus sequence as set out in SEQ ID NO: 17 and in Figure 5A;

human lambda light chain subgroup 5 consensus sequence as set out in SEQ ID NO: 18 and in Figure 5A; and wherein said plurality of heavy chain amino acid segeuences is selected from the group consisting of:

human heavy chain subgroup 3 consensus sequence as set out in SEQ ID NO: 19 and in Figure 5B;

human heavy chain subgroup 1 consensus sequence as set out in SEQ ID NO: 20 and in Figure 5B; and

human heavy chain subgroup 2 consensus sequence as set out in SEQ ID NO: 21 and in Figure 5B.

3. The method as recited in claim 2, wherein said identifying step comprises identifying those amino acids in said subject light and heavy chain sequences labelled low risk (++) in Figures 5A and 5B.

4. The method as recited in claim 2, wherein said identifying step comprises identifying those amino acids in said subject light and heavy chain sequences labeled moderate risk (0+, +0, 00) in Figures 5A and 5B.

5. A product produced by the method of claims 3 or 4.

6. The product as recited in claim 5, wherein said antibody variable domain is the H65 variable domain.

7. The product as recited in claims 5 or 6, wherein said antibody is an He3 antibody.

8. A DNA encoding the He3 antibody as recited in claim 7.

9. A vector comprising the DNA as recited in claim 8.

10. A host cell incorporating the vector as recited in claim 9.

11. A method for treating an autoimmune disease comprising the step of administering a therapeutically effective dose of an immunoglobulin reactive with CD5 to an animal having the autoimmune disease.

12. The method as recited in claim 11 wherein said animal is a human.

13. The method as recited in claim 11 wherein said autoimmune disease is selected from the group consisting of: a rheumatoid arthritis, a lupus, a psoriasis, a scleroderma, a myositis, a myocarditis, spondylarthropathies, thyroiditis, pemphigus vulgaris, diabetes mellitus type 1, progressive systemic sclerosis, aplastic anemia, myasth nia gravis,

myositis including polymyositis and dermatomyositis, Sjogren's disease, collagen vascular disease, polyarteritis, inflammatory bowel disease (including Crohn's disease and ulcerative colitis), multiple sclerosis, and primary biliary cirrhosis.

14. The method as recited in claim 11, wherein said lupus is systemic lupus erythematosis.

15. The method as recited in claim 11, wherein said therapeutically effective dose of an immunoglobulin reactive with CD5 is administered by a route selected from the group consisting: of intramuscular, intravenous, transdermal, topical, intrathecal, intraarticular, intraperitoneal, and subcutaneous routes.

16. The method as recited in claim 11, wherein said therapeutically effective dose of an immunoglobulin reactive with CD5 is administered parenterally.

17. The method as recited in claim 11, wherein said autoimmune disease is a systemic autoimmune disease.

18. The method as recited in claim 11, wherein said immunoglobulin is chimeric.

19. The method as recited in claim 11, wherein said immunoglobulin is a humanized antibody.

20. The method as recited in claim 11, wherein said immunoglobulin is a monoclonal antibody.

21. The method as recited in claim 19, wherein said humanized antibody is produced by the hybridoma having ATCC accession no. HB 11206.

22. The method as recited in claim 19 wherein the monoclonal antibody is produced by hybridoma XMMLY-H65 having A.T.C.C. Accession No. HB9286.

23. The method as recited in claim 11 wherein the therapeutically effective dose is from about 0.01 mg/kg to about 5 mg/kg of host body weight.

24. The method as recited in claim 11 wherein said therapeutically effective dose is from about 0.1 mg/kg to about 2 mg/kg of host body weight.

25. A method for treating rheumatoid arthritis comprising the step of administering a therapeutically effective dose of a monoclonal anti-CD5 antibody to a human having rheumatoid arthritis.

26. The method as recited in claim 25 wherein said monoclonal anti-CD5 antibody is produced by hybridoma XMMLY-H65 having A.T.C.C. Accession No. HB9286.

27. The method as recited in claim 25, wherein said monoclonal anti-CD5 antibody is a humanized antibody produced by hybridoma having ATCC accession no. HB 11206

28. The method as recited in claim 25, wherein said therapeutically effective dose is administered intravenously.

29. The method as recited in claim 25, wherein said therapeutically effective dose is administered parenterally.

30. The method as recited in claim 25, wherein said therapeutically effective dose is from about 0.01 mg/kg to about 5 mg/kg of said human's body weight.

31. The method as recited in claim 25, wherein the therapeutically effective dose is from about 0.1 mg/kg to about 2 mg/kg of said human's body weight.

32. The method as recited in claim 25 wherein said monoclonal anti-CD5 antibody is a human engineered H65 antibody.

33. A pharmaceutical composition comprising an immunoglobulin reactive with CD5 in a pharmaceutically acceptable diluent or carrier.

34. The pharmaceutical composition as recited in claim , 33 further comprising an immunosupressive agent, a potentiator, or a side-effect relieving agent.

35. The pharmaceutical composition as recited in claim 33 further comprising prednisolone, prednisone, dexamethasone, cyclophosphoamide, cyclosporine, methotrexate, azathiopine, or gamma-globulin.

36. The pharmaceutical composition as recited in claim 33 further comprising an angiogenesis inhibitor.

37. The pharmaceutical composition as recited in claim 33 in lyophilized form.

38. The pharmaceutical composition as recited in claim 33 in an aqueous, aerosol, or lyposomal preparation.

39. The pharmaceutical composition as recited in claim 33, wherein said immunoglobulin comprises a product according to claim 4 or claim 5.

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LIGHT CHAIN

POS		10	20 x	30x 40	50
HYH	DIVLTQS	PATLSVTPGNSVSLSCRASQS		IG NNLHWYQQKSHESPRLLIK	YAS
MCPC	DIVMTQS	PSSLSVSAGERVTMSCKSSQS		LL!NFLAWYQQKPGQPPKLLIY	GAS
NEWM	XSVLTQ	PPSVSGAPGQRVTISCTGSSSN		IG!NHVKWYQQLPGTAPKLLIF	
KOL	QSVLTQ	PPSASGTPGQRVTISCSGTSSN		IGSSTVNWYQQLPGMAPKLLIY	RAD
BIND	+-+O+++	O++++++O+++++++-+-O---		------O-O+++O+++O+-OO-	---
BURY	+-+-+-+	O++O+-+++++-+-+-+-+++-		-++++-=-=O=++++O=O=--O	+++
RISK	●■●■●■●	▲●●▲●■●▲●●■●■●■●■▲■■■		■■■■■■■■■▲■●▲●●▲■▲■■■■	■■■
MOD	· · · ·	·· · · ··· · · ·		· ··	

POS	60	70	80 90	x		100
HYH	QSISGIPSRFSGSG	SGT	DFTLSINSVETEDFGMYFCQQS	NS	WPYT	FGGGTKLDIK
MCPC	TRESGVPDRFTGSG	SGT	DFTLTISSVQAEDLAVYYCQND	HS	YPLT	FGAGTKLEIK
NEWM	HNNA RFSVSK	SGS	SATLAITGLQAEDEADYYCQSY	DR	SLRV	FGGGTKLTVL
KOL	MRPSGVPDRFSGSK	SGA	SASLAIGGLQSEDETDYYCAAW	DV!	NAYV	FGTGTKVTVL
BIND	-+OO++O++++-+-	+O+	+-+++++++++++++++-O--	---	---O	O+++++++++
BURY	++O++-O+O-+-+-	+-+	+-+-+-++-+++-+---=-=-=	++O	OO=-	=-+--+-+++
RISK	■●▲▲●■▲●▲■●■●■	●■●	●■●■●■●●■●●●■●■■■■■■■■	■■■	■■■■	■■●■■●■●●●
MOD	· · · · ·	· ·	· · · ·· ··· ·			· · ···

FIGURE 1A

HEAVY CHAIN

```
POS              10         20         30         40         50  x
HYH     DVQLQESGPS LVKPSQTLSLTCSVTG DSITSDYWSWIRKFPGNRLEYMGYVS YSGST
MCPC    EVKLVESGGG LVQPGGSLRLSCATSG FTFSDFYMEWVRQPPGKRLEWIAASR!NKYTT
NEWM    QVQLEQSGPG LVRPSQTLSLTCTVSG TSFDDYYSTWVRQPPGRGLEWIGYVF YHGTS
KOL     EVQLVQSGGG VVQPGRSLRLSCSSSG FIFSSYAMYWVRQAPGKGLEWVAIIWDDGSDQ
BIND    o-+o++++o+ +++o+++++++-+oo- --------o-o+++o+++o+-oo---------
BURY    +-+-+-+-o+ +o+++++-+-+-+-+- -+-++o+-=-=o=++++o=o=--o-o+++o++
RISK    ▲■●■●■●■▲● ●▲●▲●●●■●■●■●▲■ ■■■■■■■■■■■▲■●▲●●▲■▲■■■■■■■■■■■■
MOD       . . .  . . . ... . . .                    . ..

POS      60         70         80  ABC       90          x100A        110
HYH     YYNPSLKSRISITRDTSKNQYYLDLNSVTTEDTATYYCANWD      GDYWGQGTLVTVSA
MCPC    EYSASVKGRFIVSRDTSQSILYLQMNALRAEDTAIYYCARNYY ! WYFDVWGAGTTVTVSS
NEWM    DTDTPLRSRVTMLVNTSKNQFSLRLSSVTAADTAVYYCARNLIA  GCIDVWGQGSLVTVSS
KOL     HYADSVKGRFTISRNDSKNTLFLQMDSLRPEDTGVYFCARDGG ! FGPDYWGQGTPVTVSS
BIND    -OOOO+O++++-+-+O++O+-++++++++++++++-O------ -----O++++++++++
BURY    +O-+O-++O-+-+-+O++O+-+-+-++-+++-+-O-=-=-=OO+O OO=OO=-+--+-+-++
RISK    ■▲■▲▲■▲●▲■●■●■●▲●●▲●■●■●■●●●■●●●■●■▲■■■■■■■■■■■ ■■■■■■■■●■■●■●■●●
MOD           .  . . . .. . . . . .. ... .                    .  . . ..
```

FIGURE 1B

FIGURE 2

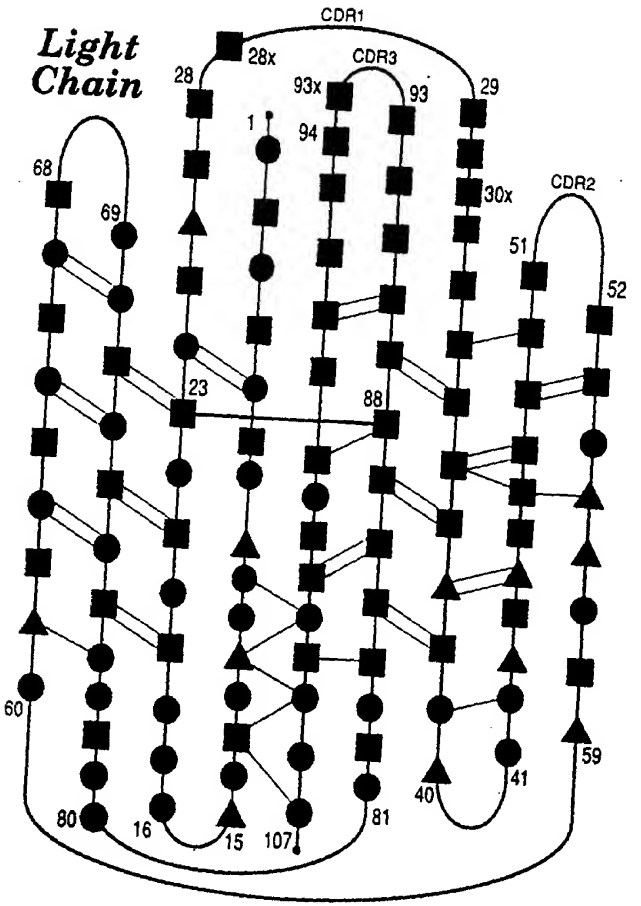
Light Chain
CDR1
28x
CDR3
93x
93
29
28
1
94
68
69
30x
CDR2
51
52
23
88
60
59
41
40
80
16
15
107
81

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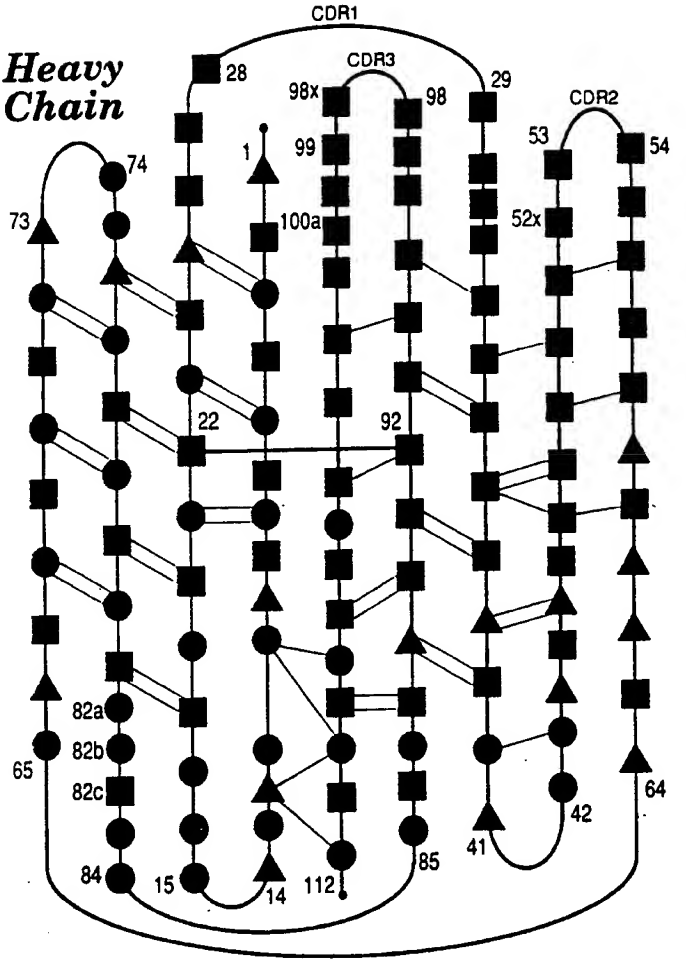
FIGURE 3


Heavy Chain
CDR1
CDR3
CDR2
28
29
98x
98
99
1
100a
53
54
52x
74
73
22
92
82a
82b
82c
65
64
42
41
84
15
14
112
85

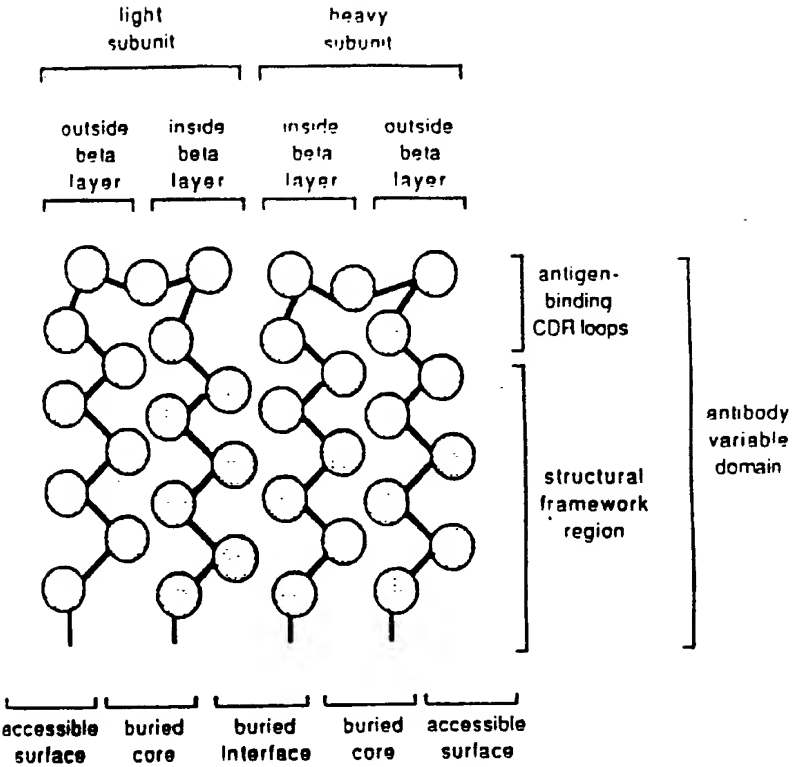

light subunit
heavy subunit
outside beta layer
inside beta layer
inside beta layer
outside beta layer
antigen-binding CDR loops
structural framework region
antibody variable domain
accessible surface
buried core
buried interface
buried core
accessible surface

Figure 4

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LIGHT CHAIN

```
POS                 10         20       x 30x        40         50
BIND     +-+o+++ o++++++o+++++++-+-o---  ------o-o+++o+++o+-oo-   ---
BURY     +-+-+-+ o++o+-+++++-+-+-+-+++-  -++++-=-=o=++++o=o=--o   +++
RISK     ●■●■●■● ▲●●▲●■●▲●●●■●■●■●■▲■■■  ■■■■■■■■■▲■●▲●●▲■▲■■■■   ■■■
MOD      . . . .  .. . . ... . . .                   . ..
HK1      DIQMTQS PSSLSASVGDRVTITCRASQx   Is xYLxWYQQKPGKAPKLLIY   AAS
HK3      EIVLTQS PGTLSLSPGERATLSCRASQS   vSSSYLAWYQQKPGQAPRLLIY   GAS
HK2      DIVMTQS PLSLPVTPGEPASISCRSSQS   LLNNYLNWYLQKPGQSPQLLIY   LGS
HL1      xSVLTQP PS ASGTPGQRVTISCSGSSS   IGxNxVxWYQQLPGTAPKLLIY   N N
HL2      XSALTQP AS VSGSPGQSITISCTGTSS   VGYNXVSWYQQHPGKAPK LIY    DV
HL3      SYELTQP PS vSVSPGQTA ITCSGDx    LxxxYVxWYQQKPGQAPVLVIY    D
HL6?     NFMLTQP HS VSESPGKTVTISCTXSXG   IASXYVQWYQQRPGSAPTTVIY   EDN
HK4?     DIVMTQS PDSLAVSLGERATINCKSSQS   VLKNYLAWYQQKPGQPPKLLIY   WAS
HL4?      SELTQP PS VSVAPGQT RITCSGDX    LGXYDAXWYQQKPGQAPLLVIY   GRN
HL5?      SALTQP PS ASGSPGQSVTISCTGTSS   VGXXYVSWYQQH G APK  I    EV
```

FIGURE 5A

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HEAVY CHAIN

```
POS               10         20         30         40         50  x
BIND   0-+0++++0+ +++0+++++++-+00- --------0-0+++0+++0+-00---------
BURY   +-+-+-+-0+ +0+++++-+-+-+-+- -+-++0+-=-=0=++++0=0=--0-0+++0++
RISK   ▲■●■●■●■▲● ●▲●▲●●●■●■●■●■▲■ ■■■■■■■■■■■■▲■●▲●●▲■▲■■■■■■■■■■■■
MOD     . . . .   . . ... . . .                 .  ..
HH3    EVQLvESGGG LVqPGGSLRLSCAASG FтFsxxxмxWVRQAPGKGLEWVxxIxxxxxGx
HH1    QVqLvqSGAE VkKPGxSvxvSCKxSG YYFxxYxIxWvRQAPGxGLEWvGxIxPxxGxT
HH2?   XVTLXESGPX LVLPTQTLTLTCTVSG XSLSXXXVXWIRQPPGKXLEWLAXIX  XDDD
```

```
POS    60        70        80  ABC     90        x100A     110
BIND   -0000+0++++-+-+0++0+-+++++++++++++++-0------ -----0++++++++++
BURY   +0-+0-++0-+-+-+0++0+-+-+-++-+++-+-0-=-=-=00+0 00=00=-+--+-+-++
RISK   ■▲■▲▲■▲●▲■●■●■●▲●●▲●■●■●■●●■●●●■●■▲■■■■■■■■■▲■ ■■■■■■■●■■●■●■●●
MOD           .   .  .  .  .  ..  .  .  .  ..  ...  .      .    .   .  ..
HH3    xYADSVkGRFTISRDDSKNтLYLQMNSLRAEDTAvYYCARxxxxx xxxxxWGQGTLVTvSS
HH1    xYAPxFQGRVTxTRDxSxNTAYMELxSLRSEDTAvYYCARxxxxx xxxxxWGQGTLvтVSS
HH2?   XYXTSLRSRLTISKDTSKNQVVLXXXXXDPXDTATYYCARXXXXX XXXDVWGQGTTVTVSS
```

FIGURE 5B

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LIGHT CHAIN

```
POS             10         20        x 30x     40          50
HK1   DIQMTQS PSSLSASVGDRVTITCRASQx  Is xYLxWYQQKPGKAPKLLIY  AAS
H65   DIKMTQS PSSMYASLGERVTITCKASQD  IN SYLSWFHHKPGKSPKTLIY  RAN
BIND  +-+O+++ O++++++O+++++++-+-O--- ------O-O+++O+++O+-OO-  ---
BURY  +-+-+-+ O++O+-+++++-+-+-+-+++- -++++-=-=O=++++O=O=--O  +++
RISK  ●■●■●■● ▲●●▲●■●▲●●●■●■●■●■▲■■■ ■■■■■■■■■▲■●▲●●▲■▲■■■■  ■■■
MOD   .  . . .  .. .  . ...  .  . .           .  ..
M/H     H       MH  M H      H    M   M M  M MMM    M       M M
PROP  DIQMTQS PSSMSASLGDRVTITCRASQD  IN SYLSWFHHKRGKSPKTLIY  RAN

POS          60         70         80         90    x        100
HK1   xLxsGVPSRFsGSG SGT xFTITLSsLQPEDFATYYCQQY xx  xPxT FGQGTKVEIK
H65   RLVDGVPSRFSGSG SGQ DYSLTISSLDYEDMGIYYCQQY DE  SPWT FGGGTKLEIK
BIND  -+OO++++O++-+- +O+ +-+++++++++++++++-O--  ---  ---O O+++++++++
BURY  ++O++-O+O-+-+- +-+ +-+-+-++-+++-+---=-=-= ++O  OO=- =-+--+-+++
RISK  ■●▲▲●■▲●▲■●■●■ ●■● ●■●■●■●●■●●●■●■■■■■■■ ■■■  ■■■■ ■■●■■●■●●●
MOD    .  .  .  .  .  .  .  .  .  ..  ...  .                 .  . ...
M/H   M MM             H MMH       HM  HMM      MM   M M    M   M
PROP  RLVDGVPSRFSGSG SGT DYTLTISSLQYEDFGIYYCQQY DE  SPWT FGGGTKLEIK
```

FIGURE 6A

HEAVY CHAIN

```
POS             10         20          30       40        50  x
hH3    EVQLvESGGG LVqPGGSLRLSCAASG FTFSXXXMXWVRQAPGKGLEWVXXIXXKXXGX
H65    QIQLVQSGPE LKKPGETVKISCKASG YTFTNYGMNWVKQAPGKGLRWMGWINTHTGEP
BIND   O-+O++++O+ +++O+++++++-+OO- --------O-O+++O+++O+-OO---------
BURY   +-+-+-+-O+ +O+++++-+-+-+-+- -+-++O+-=-=O=++++O=O=--O-O+++O++
RISK   ▲■●■●■●■▲● ●▲●▲●●●■●■●■●■▲■ ■■■■■■■■■■■■▲■●▲●●▲■▲■■■■■■■■■■■■
MOD     .  .  .   .  .  ... .  .  .                 .  ..
M/H    MM   M  MH  MM  HHMHM  H      M  MMMM M  M         M MMM MMMMMMM
PROP   QIQLVQSGPG LKKPGGSVRISCAASG YTFTNYGMNWVKQAPGKGLRWMGWINTHTGEP

POS      60        70        80  ABC      90        x100A     110
hH3    XYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCARXXXX  XXXXXWGQGTLVTVSS
H65    TYADDFKGRFAFSLETSASTAYLQINNLKNEDTATYFCTRRGYD  WYFDVWGAGTTVTVSS
BIND   -OOOO+-++++-+-+O++O+-++++++++++++++-O------  -----O++++++++++
BURY   +O-+O-++O-+-+-+O++O+-+-+-++-+++-+-O-=-=-=OO+O  OO=OO=-+--+-+-++
RISK   ■▲■▲▲■▲●▲■●■●■●▲●●▲●■●■●■●●■●●●■●■▲■■■■■■■■■■  ■■■■■■■●■■●■●■●●
MOD           .   .  .  .  ..  .  .   .  ..  ... .         .   .  .  ..
M/H    M   MM     HM MHM HM M    M H HH    M M M MMMM  MMMMM  H  M
PROP   TYADDFKGRFTFSLDTSKSTAYLQINSLRAEDTATYFCTRRGYD  WYFDVWGQGTTVTVSS
```

FIGURE 6B

SH65K-1
AGT CGT CGA CAC GAT GGA CAT GAG GAC CCC TGC TCA GTT TCT TGG CAT CCT CCT ACT
CTG GTT TCC AGG TAT CAA ATG TGA CAT CCA GAT GAC TCA GT

HUH-K1
TGA CTC GCC CGG CAA GTG ATA GTG ACT CTG TCT CCC AGA GAT GCA GAC ATG GAA GAT
GAG GAC TGA GTC ATC TGG ATG TC

HUH-K2
TCA CTT GCC GGG CGA GTC AGG ACA TTA ATA GCT ATT TAA GCT GGT TCC AGC AGA AAC
CAG GGA AAT CTC CTA AGA CCC T

HUH-K3
GAT CCA CTG CCA CTG AAC CTT GAT GGG ACC CCA TCT ACC AAT CTG TTT GCA CGA TAG
ATC AGG GTC TTA GGA GAT TTC C

HUH-K4
GGT TCA GTG GCA GTG GAT CTG GGA CAG ATT ATA CTC TCA CCA TCA GCA GCC TGC AAT
ATG AAG ATT TTG GAA TTT ATT ATT G

HUH-K5
GTT TGA TTT CAA GCT TGG TGC CTC CAC CGA ACG TCC ACG GAG ACT CAT CAT ACT GTT
GAC AAT AAT AAA TTC CAA AAT CTT C

HUH-G1
TGT CGA CAT CAT GGC TTG GGT GTG GAC CTT GCT ATT CCT GAT GGC AGC TGC CCA AAG
TGC CCA AGC ACA GAT CCA GTT GGT GCA G

HUH-G2
AAG GTA TAC CCA GAA GCT GCG CAG GAG ATT CTG ACG GAC CCT CCA GGC TTC TTC AGG
CCA GGT CCA GAC TGC ACC AAC TGG ATC T

HUH-G3
GCA GCT TCT GGG TAT ACC TTC ACA AAC TAT GGA ATG AAC TGG GTG AAG CAG GCT CCA
GGA AAG GGT TTA AGG TGG ATG GGC TGG

HUH-G4
AAA GAG AAG GTA AAC CGT CCC TTG AAG TC A TCA GCA TAT GTT GGC TCT CCA GTG
TGG GTG TTT ATC CAG CCC ATC CAC CTT AAA C

HUH-G5
CAG GGT TTA CCT TCT CTT TGG ACA CGT CTA AGT GCA CTG CCT ATT IAC AGA TCA ACA
GCC TCA GAG CCG AGG ACA CGG CTA CAT

HUH-G6
AGG AGA CGG TGA CCG TGG TCC CTT GGC CCC AGA CAT CGA AGT ACC AGT CGT AAC CCC
GTC TTG TAC AGA AAT ATG TAG CCG TGT CCT CGG C

H65G-2S
ACT AGT GTC GAC ATC ATG GCT TGG GT

H65-G2
GAG GAG ACG GTG ACC GTG GT

H65K-2S
AGT CGT CGA CAC GAT GGA CAT GAG GAC

JK1-HindIII
GTT TGA TTT CAA GCT TGG TGC

FIGURE 7A

HUH-G11
TGT CGA CAT CAT GGC TTG GGT GTG GAC CTT GCT ATT CCT GAT GGC AGC TGC CCA AAG
TGC CCA AGC AGA GAT CCA GTT GGT GCA G

HUH-G14
AAA GAG AAG GTA AAC CGT CCC TTG AAA GAA TCA GCA TAT GTT GGC TCT CCA GTG TGG
GTG TTT ATC CAG CCC ATC CAC TCT AAA C

HUH-G13
GCA GCT TCT GGG TAT ACC TTC ACA AAC TAT GGA ATG AAC TGG GTG CGC CAG GCT CCA
GGA AAG AAT TTA GAG TGG ATG GGC TGG

HUH-G16
GAG GAG ACG GTG ACC GTG GTC CCT TGG CCC CAG ACA TCG AAG TAC CAG TCG TAA CCC
CGT CTT GTA CAG AAA TAC ACA GCC GTG TCC TCG GC

HUH-G15
GAC GGT TTA CCT TCT CTT TGG ACG ATT CTA AGA ACA CTG CCT ATT TAC AGA TCA ACA
GCC TCA GAG CCG AGG ACA CGG CTG TGT ATT

HUH-G12
AAG GTA TAC CCA GAA GCT GCG CAG GAG ATT CTG ACG GAC CCT CCA GGC TTC ACC AGG
CCT CCT CCA GAC TGC ACC AAC TGG ATC TC

HUH-K6
TCA CTT GCC GGG CGA ATC AGG ACA TTA ATA GCT ATT TAA GCT GGT TCC AGC AGA AAC
CAG GGA AAG CTC CTA AGA CCC T

HUH-K8
GAT CCA CTG CCA CTG AAC CTT GAT GGG ACC CCA GAT TCC AAT CTG TTT GCA CGA TAG
ATC AGG GTC TTA GGA GCT TTC C

HUH-K7
TGA CTC GCC CGG CAA GTG ATA GTG ACT CTG TCT CCT ACA GAT GCA GAC AGG GAA GAT
GGA GAC TGA GTC ATC TGG ATG TC

FIGURE 7B

```
          10        20        30        40        50
          |         |         |         |         |
ACTAGTGTCG ACATCATGGC TTGGGTGTGG ACCTTGCTAT TCCTGATGGC
AGCTGCCCAA AGTGCCCAAG CACAGATCCA GTTGGTGCAG TCTGGACCTG
GCCTGAAGAA GCCTGGAGGG TCCGTCAGAA TCTCCTGCGC AGCTTCTGGG
TATACCTTCA CAAACTATGG AATGAACTGG GTGAAGCAGG CTCCAGGAAA
GGGTTTAAGG TGGATGGGCT GGATAAACAC CCACACTGGA GAGCCAACAT
ATGCTGATGA CTTCAAGGGA CGGTTTACCT TCTCTTTGGA CACGTCTAAG
AGCACTGCCT ATTTACAGAT CAACAGCCTC AGAGCCGAGG ACACGGCTAC
ATATTTCTGT ACAAGACGGG GTTACGACTG GTACTTCGAT GTCTGGGGCC
AAGGGACCAC GGTCACCGTC TCCTC
```

FIGURE 8A

```
        10         20         30         40         50
         |          |          |          |          |
AGTCGTCGAC ACGATGGACA TGAGGACCCC TGCTCAGTTT CTTGGCATCC
TCCTACTCTG GTTTCCAGGT ATCAAATGTG ACATCCAGAT GACTCAGTCT
CCATCTTCCA TGTCTGCATC TCTGGGAGAC AGAGTCACTA TCACTTGCCG
GGCGAGTCAG GACATTAATA GCTATTTAAG CTGGTTCCAG CAGAAACCAG
GGAAATCTCC TAAGACCCTG ATCTATCGTG CAAACAGATT GGTAGATGGG
GTCCCATCAA GGTTCAGTGG CAGTGGATCT GGGACAGATT ATACTCTCAC
CATCAGCAGC CTGCAATATG AAGATTTTGG AATTTATTAT TGTCAACAGT
ATGATGAGTC TCCGTGGACG TTCGGTGGAG GCACCAAGCT TGAAATCAAA
```

FIGURE 8B

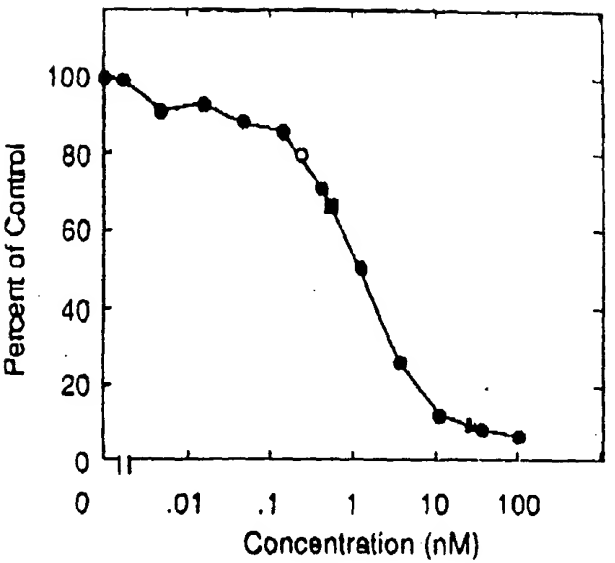
Percent of Control
100
80
60
40
20
0
0
.01
.1
1
10
100
Concentration (nM)

Figure 9

LIGHT CHAIN

```
POS            10         20        x 30x      40            50
EU     DIQMTQS PSTLSASVGDRVTITCRASQS  IN TWLAWYQQKPGKAPKLLMY  KAS
HK1    DIQMTQS PSSLSASVGDRVTITCRASQx  Is sYLxWYQQKPGKAPKILIY  AAs
TAC    QIVLTQS PAIMSASPGEKVTITCSASSS  IS  YMHWFQQKRGTSPKLWIY  TTS
BIND   +-+O+++ O++++++O+++++++-+-O--- ------O-O+++O+++O+-OO-  ---
BURY   +-+-+-+ O++O+-+++++-+-+-+-+++- -++++-=-=O=++++O=O=--O  +++
RISK   ●■●■●■● ▲●●▲●■●▲●●●■●■●■●■▲■■■ ■■■■■■■■■▲■●▲●●▲■▲■■■■  ■■■
MOD    .  .  .  .  ..  .  . ...  .  .  .       .  ..
M/H    H HM     HHM   M HH     H    M     M MM M     HM       MM
PROP   DIQLTQS PSSMSASPGDRVTITCRASSS  IS  YMHWFQQKPGKSPKLWIY  TTS
QUE    DIQMTQS PSTLSASVGDRVTITCSASSS  IS  YMHWYQQKPGKAPKLLIY  TTS

POS           60       70      80         90    x      100
EU     SLESGVPSRFIGSG SGT EFTLTISSLQPDDFATYYCQQY NS  DSKM FGQGTKVEVK
HK1    xLxsGVPSRFsGSG SGT xFTLTISsLQpEDFATYYCQQY XX  xPxT FGQGTKVEIK
TAC    NLASGVPARFSGSG SGT SYSLTISRMEAEDAATYYCHQR ST  YPLT FGSGTKLELK
BIND   -+OO++O++++-+- +O+ +-+++++++++++++++-O--  --- ---O O+++++++++
BURY   ++O++-O+O-+-+- +-+ +-+-+-++-+++-+---=-=-= ++O OO=- =-+--+-+++
RISK   ■●▲▲●■▲●▲■●■●■ ●■● ●●●■●●●■●●●●■●■■■■■■■■ ■■■ ■■■■ ■■●■■●■●●●
MOD     .  .  .  .  .  . .  .  .  .  .. ...  .             .  . ...
M/H    M M    H      MMH          HMHM  H     M M MM  M M   H   M  M
PROP   NLASGVPSRFSGSG SGT SYTLTISSMQAEDFATYYCHQR ST  YPLT FGQGTKLELK
QUE    NLASGVPARFSGSG SGT EFTLTISSLQPDDFATYYCHQR ST  YPLT FGQGTKVEVK
```

FIGURE 10A

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HEAVY CHAIN

```
POS              10          20          30       40        50  X
EU      QVQLVQSGAE VKKPGSSVKVSCKASG GTFSRSAIIWVRQAPGQGLEWMGGIVPMFGPP
hH1     QVqLvqSGaE VkKPGxSvxvSCKxSG yyFxxyxIxWvRQaPGxGLEWvGxIxpxxGxT
TAC     QVQLQQSGAE LAKPGASVKMSCKASG YTFTSYRMHWVKQRPGQGLEWIGYINPSTGYT
BIND    o-+o++++o+ +++o+++++++-+oo- ---------o-o+++o+++o+-oo---------
BURY    +-+-+-+-o+ +o+++++-+-+-+-+- -+-++o+-=-=o=++++o=o=--o-o+++o++
RISK    ▲■●■●■●■▲● ●▲●▲●●●■●■●■●■▲■ ■■■■■■■■■■■▲■●▲●●▲■▲■■■■■■■■■■■■
MOD      .  .  .   .  .  .  ... .  .  .         .  ..
M/H         M       HM   M  MM    M     M MM MMM   M H  M      M M M MM M
PROP    QVQLQQSGAE VAKPGASVKMSCKASG YTFTSYRMHWVKQAPGQGLEWIGYINPSTGYT
QUE     QVQLVQSGAE VKKPGSSVKVSCKASG TYFTSYRMHWVRQAPGQGLEWIGYINPSTGYT

POS      60       70        80 ABC    90        X100A      110
EU      NYAQKFQGRVTITADESTNTAYMELSSLRSEDTAFYFCAG GYGI  YSPEEYNGGLVTVSS
hH1     xyapxfqgRVTxtrdxSxntayMeLxsLrseDtAvYYCArxxxxx  xxxxwgqGtlvtVSS
TAC     EYNQKFKDKATLTADKSSSTAYMQLSSLTFEDSAVYYCAR GGGV   FDYWGQGTTLTVSS
BIND    -oooo+o++++-+-+o++o+-++++++++++++++++-o------ -----o+++++++++
BURY    +o-+o-++o-+-+-+o++o+-+-+-++-+++-+-o-=-=-=oo+o oo=oo=-+--+-+-++
RISK    ■▲■▲▲■▲●▲■●■●■●▲●●▲●■●■●■●●■●●●■●■▲■■■■■■■■■■ ■■■■■■■●■■●■●■●●
MOD          .   .  .  .  .. .  .  .  .. ...  .                 .    .  .  ..
M/H     M MMM MhMM M M M mM    H M  HH  H          MMMM   MMM      M
PROP    EYNQKFKGKATLTADKSSSTAYMELSSLRSEDTAVYYCAR GGGV   FDYWGQGTTLTVSS
QUE     EYNQKFKDKATITADESTNTAYMELSSLRSEDTAVYYCAR GGGV   FDYWGQGTLVTVSS
```

FIGURE 10B

		Kd-1I (nM)	Kd-2I (nM)	Receptors/cell
cH65 IgG		3.8		23,046
HE3 IgG	12G9 Supernatant	3.8	1,020	80,668

FIGURE 11

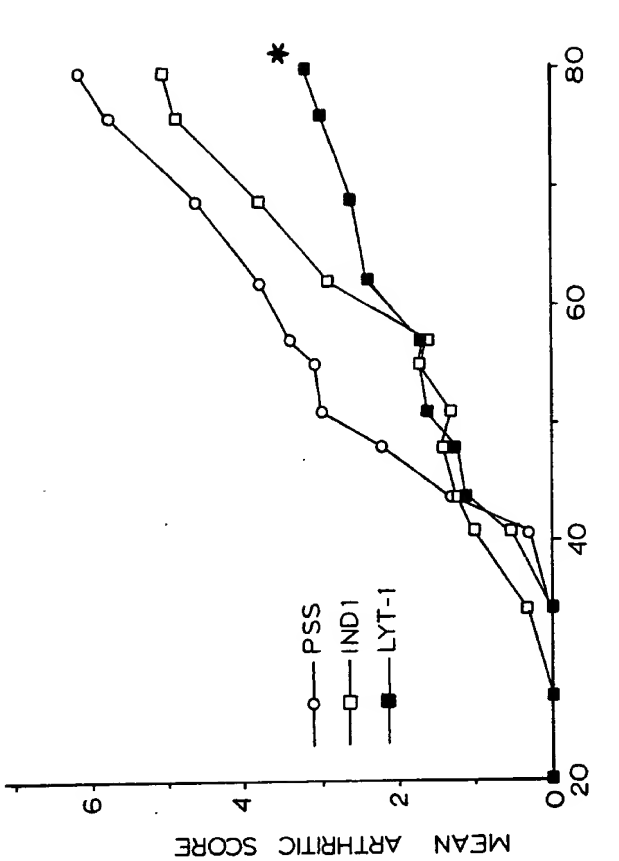

EFFECT OF ANTI-LYT-1 ON COLLAGEN-INDUCED ARTHRITIS
MEAN ARTHRITIC SCORE
0
2
4
6
20
40
60
80
DAY AFTER BII IMMUNIZATION
PSS
IND1
LYT-1
*

FIG.12

FIGURE 13A

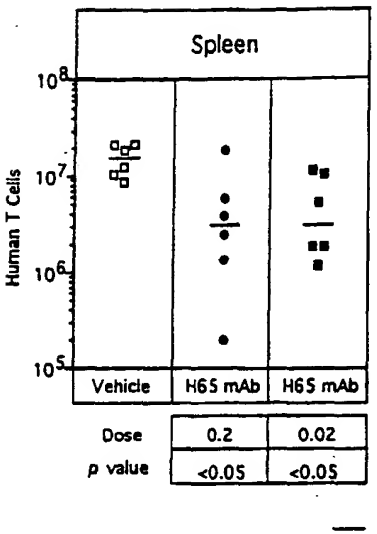
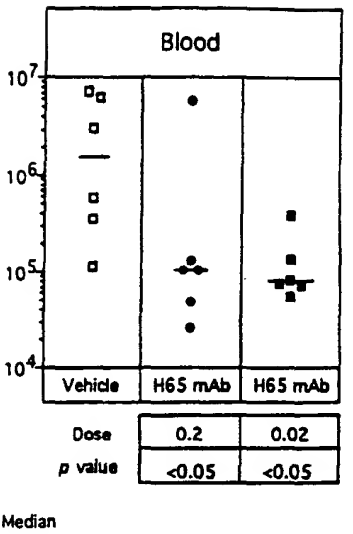


FIGURE 13B



— Median

FIGURE 14A

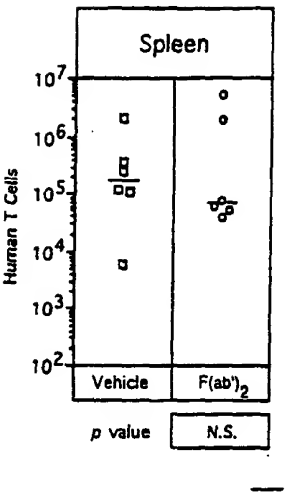
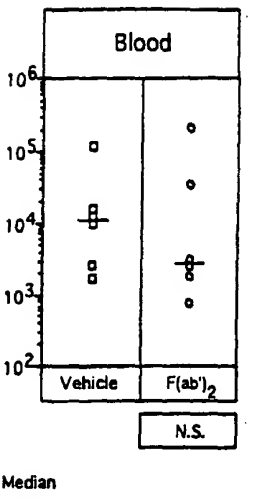


FIGURE 14B



— Median

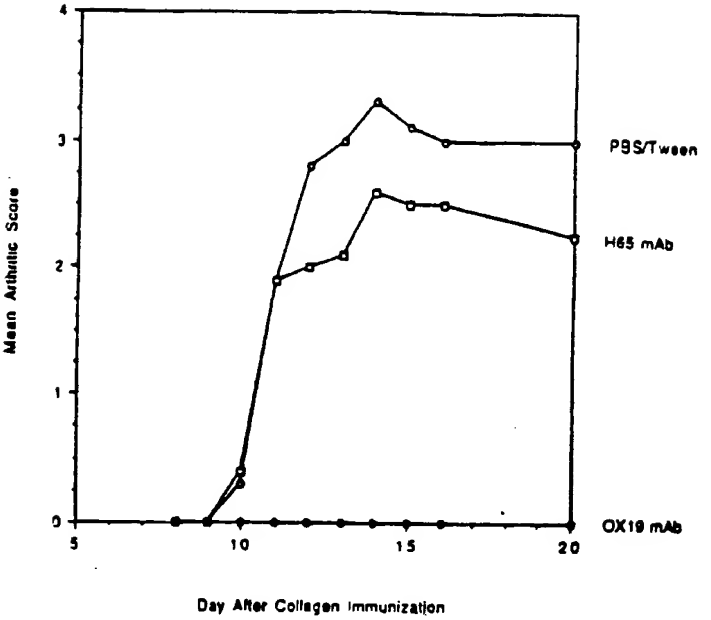

Mean Arthritic Score
0
1
2
3
4
5
10
15
20
Day After Collagen Immunization
PBS/Tween
H65 mAb
OX19 mAb

FIGURE 15

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LIGHT CHAIN

```
POS              10        20        x 30x        40           50
H65      DIKMTQS PSSMYASLGERVTITCKASQD  IN SYLSWFQQKPGKSPKTLIY   RAN
BIND     +-+O+++ O++++++O+++++++-+-O---  ------O-O+++O+++O+-OO-   ---
BURY     +-+-+-+ O++O+-+++++-+-+-+-+++-  -++++-=-=O=++++O=O=--O   +++
RISK     ●■●■●■● ▲●●▲●■●▲●●●■●■●■●■▲■■■  ■■■■■■■■■■▲■●▲●●▲■▲■■■■   ■■■
hK1      DIQMTQS PSSLSASVGDRVTITCrASQx  Is xyLxWYQQKPGkAPklLIY   aAS
M/H        ^        H^  H ^     ^    M   M M  M         H  M     M M
PROP     DIQMTQS PSSLSASVGDRVTITCRASQD  IN SYLSWFQQKPGKAPKTLIY   RAN

POS          60           70        80            90   x      100
H65      RLVDGVPSRFSGSG SGQ DYSLTISSLDYEDMGIYYCQQY DE  SPWT FGGGTKLEIK
BIND     -+OO++O++++-+-  +O+ +-++++++++++++++-O--  ---  ---O O+++++++++
BURY     ++O++-O+O-+-+-  +-+ +-+-+-++-+++-+---=-=-= ++O  OO=- =-+--+-+++
RISK     ■●▲▲●■▲●▲■●■●■  ●■● ●■●■●■●●■●●●■●■■■■■■■■ ■■■  ■■■■ ■■●■■●■●●●
hK1      xLxsGVPSRFsGSG SGT xFTlTISsLQpeDfATYYCqqy xx  xPxt FGqGTkveik
M/H      M hh              ^ ^M^      ^^   ^MM       MM  M M    ^    M
PROP     RLESGVPSRFSGSG SGT DYTLTISSLQYEDFGIYYCQQY DE  SPWT FGGGTKLEIK
```

FIGURE 16A

HEAVY CHAIN

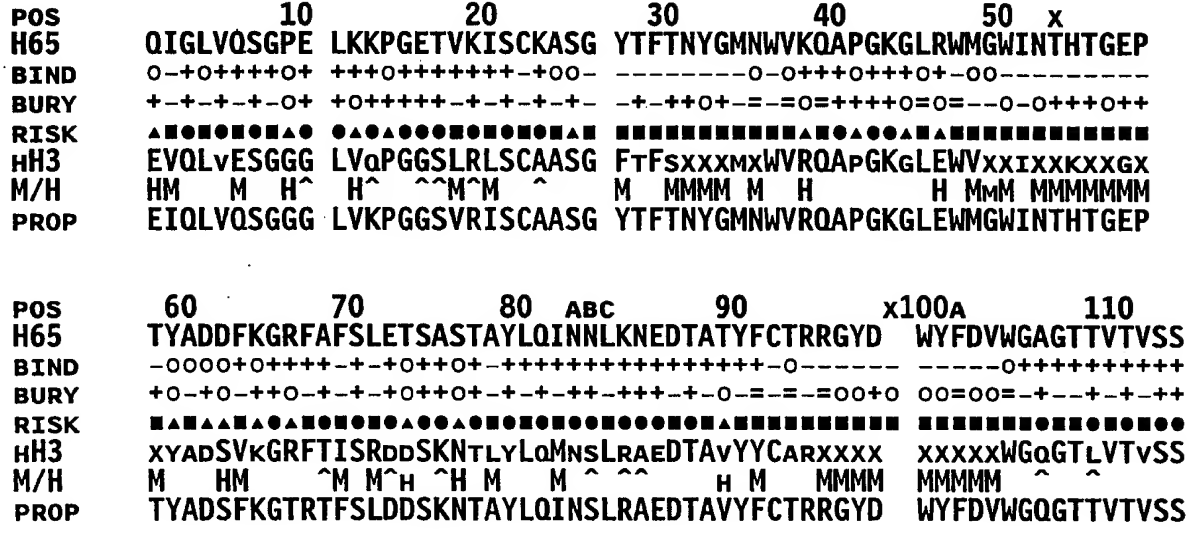

FIGURE 16B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/10906

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 39/395, 39/40; C07K 3/00, 13/00, 15/28; C12P 21/08
US CL :424/85.8; 530/387.1, 387.3; 435/69.6, 69.1, 69.7, 320.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/85.8; 530/387.1, 387.3; 435/69.6, 69.1, 69.7, 320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, MEDLINE, BIOSIS, EMBASE, WPI
search terms: studnicka, antibod?, variable, hypervariable, conformation, model?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US,A, 4,946,778 (Ladner et al.) 07 August 1990, see entire document.	1-39
Y	Nature, Volume 342, issued 21 December 1989, C.Chothia et al., "Conformations of Immunoglobulin Hypervariable Regions", pages 877-883, see entire document.	1-39
Y	Proc. Natl. Acad. Sci., Volume 86, issued December 1989, A.C.R.Martin et al., "Modeling Antibody Hypervariable Loops: A Combined Algorithm", pages 9268-9272, see entire document.	1-39
Y	Biochem. J., Volume 252, issued June 1988, G.M.Studnicka, "Escherichia coli Promoter -10 and -35 Region Homologies Correlate with Binding and Isomerization Kinetics", pages 825-831, see entire document.	1-39
Y	Cancer Research, Volume 50, issued 01 March 1990, R.P. Junghans	6-39

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 12 MARCH 1993	Date of mailing of the international search report 22 MAR 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer PHILLIP GAMBEL Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/10906

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Blood, Volume 75, Number 7, issued 01 April 1990, V.S. Byers et al., "Use of an Anti-Pan T-Lymphocyte Ricin A Chain Immunotoxin in Steroid-Resistant Acute Graft-Versus-Host Disease", pages 1426-1432, see entire document.	6-39